Universidade de Aveiro Departamento de Química

Ano: 2012

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Fotodegradação do retardador de chama BDE-209.

Photodegradation of BDE-209 flame retardant.

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Química Analítica e Qualidade, realizada sob a orientação científica da Doutora Maria Eduarda Bastos Henriques dos Santos e do Doutor Valdemar Inocêncio Esteves, Professores Auxiliares do Departamento de Química da Universidade de Aveiro.

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Palavras-chave BDE-209, retardadores de chama, fotodegradação, água, substâncias húmicas, HPLC, DLLME, SPE

Resumo

O BDE-209 ou decaBDE é o retardador de chama mais bromado e é usado em diversos materiais tais como plásticos, têxteis e madeira para melhorar a resistência ao fogo. Este retardador de chama é já considerado um contaminante prioritário e pode ser encontrado em diversas matrizes ambientais, nomeadamente, em água. Apesar dos baixos níveis encontrados em água, o decaBDE tende a acumular-se em peixes, por exemplo. A toxicidade associada à exposição crónica a longo prazo já foi provada no modelo *Zebrafish*, mesmo a baixos níveis de concentração de BDE-209. A fotodegradação é uma importante via de degradação de contaminantes em águas naturais e essa fotodegradação pode, por um lado, remover os contaminantes do ambiente, mas, por outro lado, os produtos resultantes podem ser mais tóxicos do que o composto original.

O objetivo principal deste trabalho foi conduzir o estudo da fotodegradação do BDE-209 em água em concentrações ambientalmente relevantes (5 µg/L), sob a ação de luz solar simulada, e avaliar a influência de substâncias húmicas. Até à data, não são conhecidos estudos da fotodegradação do BDE-209 em solução aquosa, mas há registo de que a natureza do solvente assume uma grande influência na sua fotodegradação. Assim, as mesmas experiências foram realizadas em etanol para se comparar o comportamento do BDE-209 em ambos os solventes, com as mesmas condições de irradiação.

O estudo da fotodegradação de BDE-209 em água foi considerado importante mas praticamente impossível por alguns autores, devido aos problemas analíticos levantados pela reduzida solubilidade do composto e pela sua tendência para adsorver às paredes dos recipientes. Assim, o presente trabalho foi um desafio em termos do desenvolvimento de metodologias analíticas para a análise do BDE-209 e a deteção de fotoprodutos ao longo da irradiação. Duas metodologias foram testadas para a pré-concentração do BDE-209: micro-extração dispersiva líquido-líquido (sigla inglesa DLLME) e extração em fase sólida e vários detalhes experimentais foram otimizados para controlar fontes de contaminação e aumentar a reprodutibilidade.

Os resultados permitiram as seguintes conclusões: a fotodegradação do BDE-209 foi mais lenta em água do que em etanol; foram detetados mais produtos de fotodegradação em água do que em etanol; as substâncias húmicas promovem o atraso da fotodegradação do BDE-209, principalmente em solução aquosa. Foram também feitas diversas considerações sobre possíveis vias de degradação. Adicionalmente foi conseguido um LOD de 523 ng/L com o procedimento de pré-concentração e análise em amostras aquosas DLLME – HPLC – UV.

Com os resultados obtidos, um artigo foi submetido à *Chemosphere* e está em processo de revisão. Uma comunicação oral e um poster foram apresentados num encontro nacional e internacional, respetivamente.

Keywords BDE-209, flame retardants, photodegradation, water, humic substances, HPLC, DLLME, SPE

Abstract

BDE-209 or decaBDE is the most brominated flame retardant and it is used in several materials such as plastics, textiles and wood to improve resistance to fire. This flame retardant is considered a priority contaminant and can be found in several environmental matrices, namely, in water. Despite the low levels found in water, decaBDE tends to accumulate in fish, for example. The toxicity associated to chronic exposure to low doses of BDE-209 was already proved in the *Zebrafish* model. Photodegradation is an important degradation pathway of contaminants in natural waters and the photodegradation of contaminants can, on one hand, remove them from the environment but, on the other hand, photoproducts may be more toxic than the parent compound.

The main goal of this work was to conduct a study of the BDE-209 photodegradation in water, at concentrations environmentally relevant (5 µg/L), under simulated solar radiation and to assess the influence of humic substances. To date, no studies are known on the photodegradation of BDE-209 in aqueous solution, but it is documented that the solvent nature has a marked influence on its photodegradation. Thus, the same experiments were done in ethanol to compare the BDE-209 behaviour in both solvents, with the same irradiation conditions.

The study of photodegradation of BDE-209 in water has been considered important but practically impossible by some authors, due to the analytical problems raised by the low solubility of the compound and its tendency to adsorb to flasks walls. Thus, the present work was a challenge in terms of the development of analytical methodologies for the analysis of BDE-209 and detection of photoproducts along irradiation. Two methodologies were tested for the pre-concentration of BDE-209: dispersive liquid-liquid micro-extraction (DLLME) and solid phase extraction (SPE) and several experimental details were optimized in order to control sources of contamination and improve reproducibility.

The results allowed the following conclusions: BDE-209 photodegradation in water was slower than in ethanol; more photoproducts were detected in water than in ethanol; the humic substances promote the delay of BDE-209 photodegradation, mainly in aqueous solution. Considerations about possible degradation pathways are also included. In addition a LOD of 523 ng/L was achieved with the procedure of pre-concentration and analysis in aqueous samples DLLME – HPLC – UV.

With these results, an article was submitted to Chemosphere and is under review, and one oral communication and one poster were presented in one national and one international meeting, respectively.

Abbreviations

ATSDR – Agency for Toxic Substances and Disease Registry

BFR – Brominated Flame-retardant

BSEF – Bromine Science and Environmental Forum

DLLME – Dispersive Liquid-Liquid Microextraction

DOC – Dissolved Organic Carbon

EBFRIP – European Brominated Flame Retardant Industry Panel

EFRA – European Flame-retardants Association

EHC – Environmental Health Criteria

EPA – Environmental Protection Agency

FA – Fulvic Acids

FR – Flame Retardant

HA – Humic Acids

HBCD – Hexabromocyclododecane

HPLC – High Performance Liquid Chromatography

HS – Humic Substances

IRIS – Integrated Risk Information

LOD – Limit of Detection

MSDS – Material Safety Data Sheet

NOAEL – No Observable Adverse Effect Level

PBB – Polybrominated biphenyls

PBDDs – Polybrominated Dibenzo-p- Dioxins

PBDE – Polybrominated Diphenyl Ether

PBDFs – Polybrominated DibenzoFurans

PCB – Polychlorinated biphenyls

RoHS – Restriction of Hazardous Substances

SPE – Solid Phase Extraction

TBBPA – Tetrabromobisphenol A

WHO – World Health Organization

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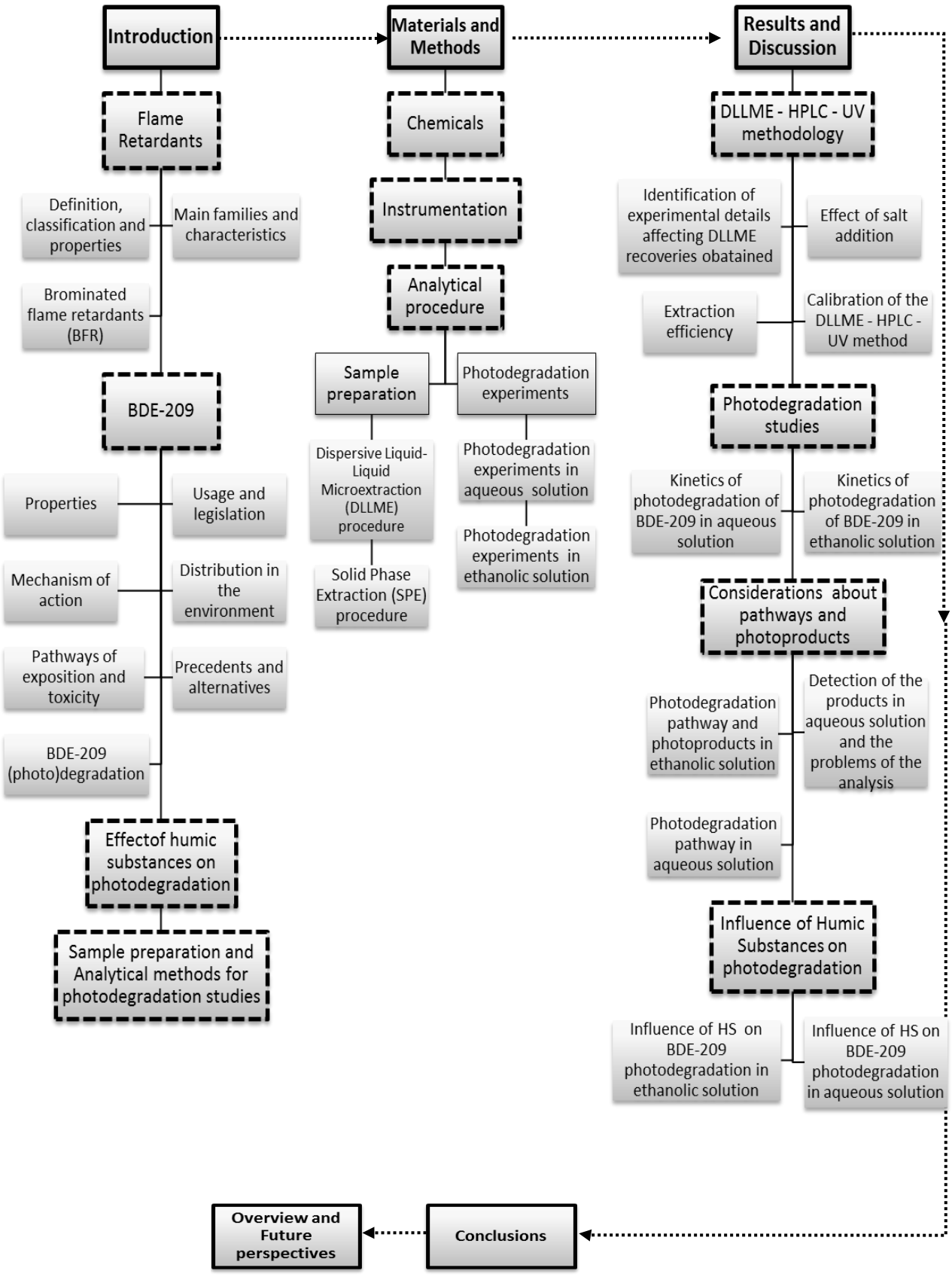
Work structure



Introduction

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1. Flame-retardants (FR)

Definition, classification and properties

Flame-retardants (FR) are chemicals added to combustible materials such as plastics, textiles and wood in order to improve the properties of resistance to fire (Pestana *et al.* 2008). The use of flame-retardants inhibits the ignition of consumer products and building materials. Several studies performed by companies responsible for development of FR and independent fire safety authorities showed the advantages of the application of these chemicals on combustible materials. Some of these advantages and characteristics are presented in figure 1 (EFRA 2007).

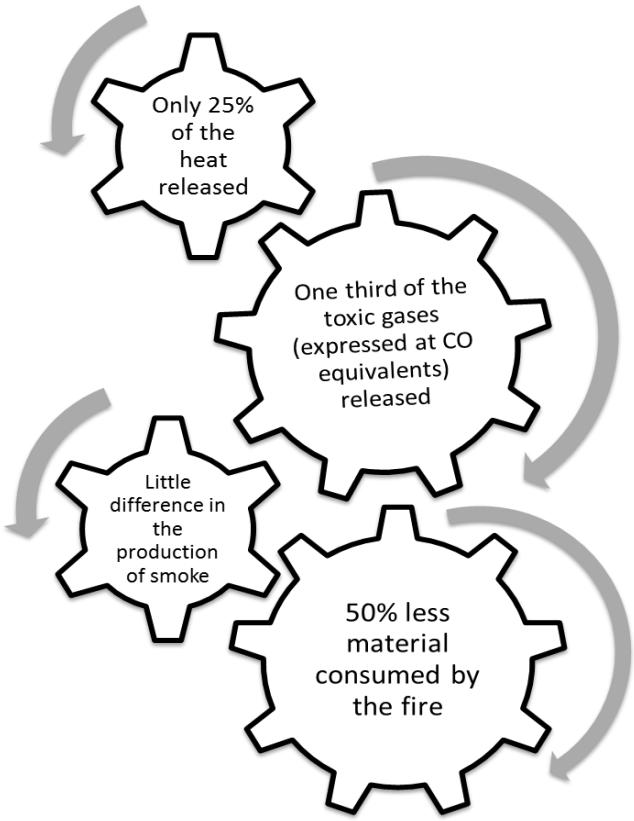


Figure 1 – Main advantages/characteristics of the application of FR on combustible materials (EFRA 2007)

The properties of resistance to fire can be improved either by physical mechanisms - cooling, formation of a protective layer and / or dilution - or by chemical mechanisms - reaction in the gas phase and / or reaction in the solid phase (EHC192 1997). Thus,

according to WHO, it is possible to distinguish five main steps that characterize the performance of systems for fire retardancy of organic polymers (EPA[a] ; EHC192 1997):

- Dilution gas – requires the use of additives that produce large volumes of non-combustible gases when the product decomposes during combustion and the gases dilute the oxygen supply to the flame – metal hydroxides, metal carbonates and some nitrogen producing compounds work in this way when used as flame retardants;
- Thermal quenching – results from the thermal decomposition of flame retardant – metal hydroxides and carbonates act in this way;
- Protective coating – some flame retardants work by forming a protective liquid or char barrier that acts as an insulating layer to reduce the heat transfer from the flame to the combustible product – phosphorous compounds that decompose to give phosphoric acid and intumescent systems operate by this mechanism;
- Physical dilution – the flame retardant can act as a thermal sink, increasing the heat capacity of the product or reducing the fuel content to a level below the lower limit of flammability – inert fillers such as glass fibres and microspheres and minerals such as talc act by this mechanism;
- Chemical interaction - some flame-retardants (halogenated and some phosphorous compounds) dissociate into radical species that compete with the propagation steps of chain reactions that occur during the combustion process – this is the general mechanism by which brominated flame-retardants operates.

Several steps may occur sequentially or in combination (EHC192 1997; Morf *et al.* 2003).

A distinction must be done between reactive and additive flame-retardants. Reactive flame-retardants are reactive components chemically built into a polymer molecule. They are added during the polymerization process and become an integral part of the polymer. The result is a modified polymer with flame retardant properties and different molecular structure compared to the original polymer molecule. Additive flame-retardants (e.g. decaBDE) are incorporated into the polymer either prior to, during or (most frequently) following polymerization (EHC192 1997). This type of FR are monomer molecules that are not covalently bound to the polymer, therefore, they can be released from the polymer to the environment (SFT 2009). In this case, the retardant and the polymer are just physically mixed together, which makes easier to manufacture of this type

of flame-retardants. Ideally, a flame retardant additive should be easy to mix, be stable to heat and light, colourless, efficient, inexpensive. On the other hand, it should not affect the physical properties of the polymer, and this is the major limitation of this type of flame retardant. The problem associated with these compounds relates to their tendency to be a fugitive, that is, to show a great propensity for leaching compared with the reactive FR (EHC162 1994).

Just out of curiosity, do not think that this concern in order to reduce the flammability of materials is a current trend, far from it. There are records of about 450 BC reporting the use of aluminium by the Egyptians to reduce the flammability of wood. The recent and accelerated development and application of a variety of flame retardants was a result of the increasing industrialization, particularly the growing production and consumption of products made of polymeric materials.

Main families and characteristics

The various families of flame-retardants are organized according to four main groups: inorganic, halogenated organics, organophosphates and nitrogen-based compounds. The first three groups are particularly relevant. Each of these families is subdivided, in turn, into a variety of other sets of compounds grouped according to figure 2 (EHC192 1997).

Some of the most common examples associated to each of the families presented are the aluminium tri-hydroxide, magnesium hydroxide and antimony trioxide for inorganic compounds; brominated and chlorinated compounds for halogenated organics; phosphate esters for organophosphates. Focusing attention on the predominance of commercial use of each of the families it is evident the limited use of nitrogen-based FRs. Contrary to this trend, the inorganic FR are the most widely used

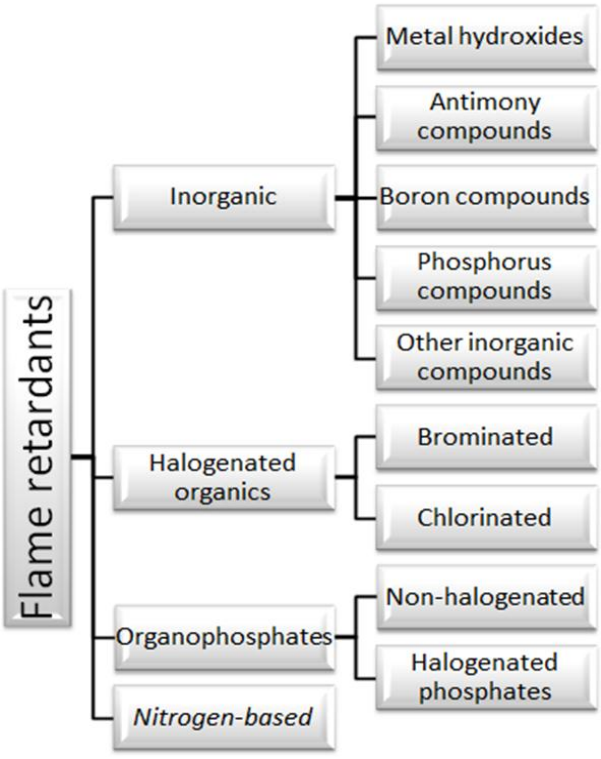


Figure 2 – Main groups of FR

commercially, followed by halogenated organics FR and organophosphates. It is not easy to find estimates of world production for each family of flame-retardants, however, 1994 records pointed to a volume percentage, globally, of 50%, 25% and 20% for inorganic flame-retardants, halogenated organics and organophosphates, respectively (EHC192 1997).

Brominated flame-retardants (BFR)

According to figure 3, the brominated flame-retardants are grouped in HBCD (Hexabromocyclododecane), TBBPA (Tetrabromobisphenol A), PBDDs / PBDFs (Polybrominated Dibenzo-p-Dioxins / Polybrominated DibenzoFurans), PBBs (Polybrominated biphenyls) and PBDEs (Polybrominated Diphenyl Ethers) (Xie[b] and Ebinghaus 2008; Tadeo *et al.* 2012). With the exception of PBDDs / PBDFs, PBBs and some PBDEs that are already banned, all other classes of BFRs are subject to tight surveillance actions. In addition, there is one classification more recent: the novel brominated flame retardants (NBFRs) which have been introduced to replace the BFRs banned. Decabromodiphenyl ethane (DBDPE or deBDethane) appears as mainly alternative to BDE-209 due to its similar physicochemical properties. Furthermore, DBDPE is slightly more hydrophobic and less sensitive to thermal degradation than BDE-209. Other examples of these novel brominated flame retardants are 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) to replace the banned octa-BDE, or TBBPA derivatives (Tadeo *et al.* 2012).

Data from the European Flame Retardants Association (EFRA 2006) show that the total European consumption of brominated flame retardant estimated in 2005 was 50000 tonnes for a total of 463 800 tonnes (10.8 %). Only the flame retardant aluminium hydroxide exceeded this amount with a percentage of 49.6 %. Of all brominated flame-retardants, TBBPA and PBDEs are the most commonly used.

PBDEs are a group of 209 congeners (annex 1) and, among all of them, three stand out for their wide commercial use – pentaBDE, octaBDE and decaBDE, assuming the latter, the greatest relevance to this work.

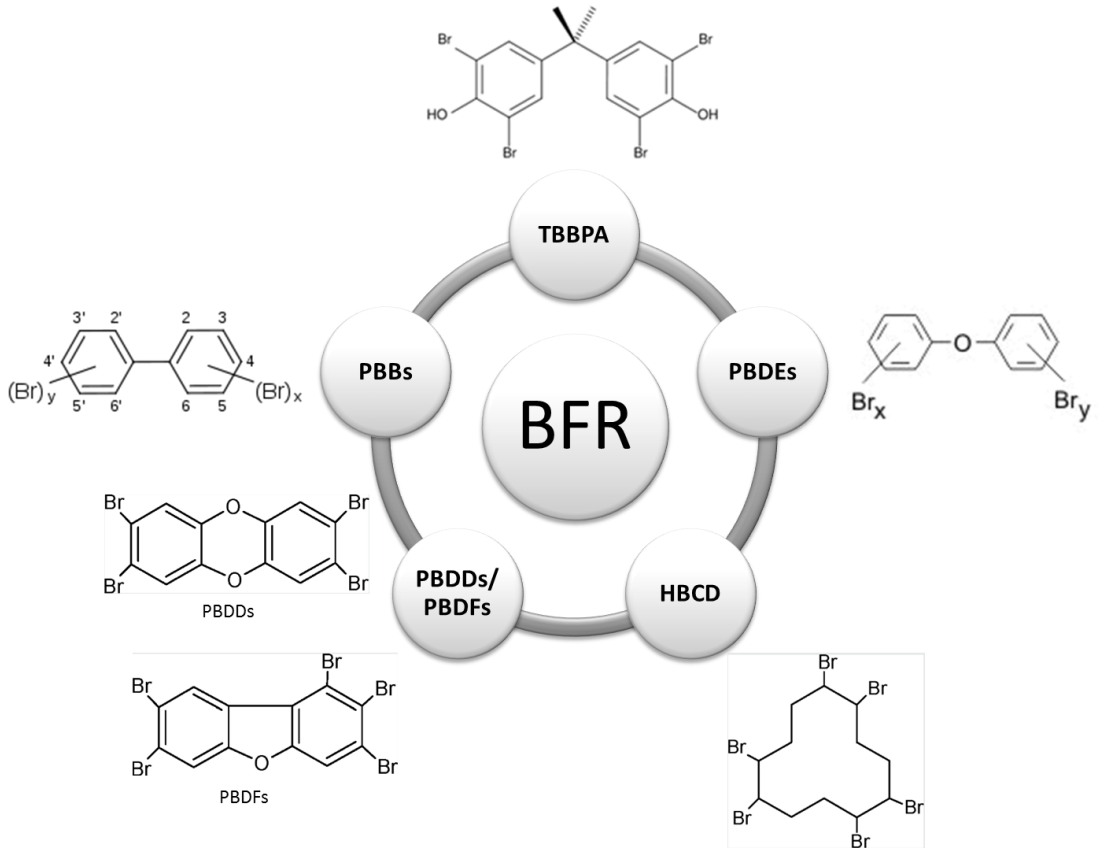


Figure 3 – Brominated flame-retardants

2. BDE-209

Properties

The 2,2',3,3',4,4',5,5',6,6' – Decabromodiphenyl ether (figure 4) is known as decaBDE, BDE-209 or bis(pentabromphenyl) ether (IUPAC name) and it is the most brominated flame retardant. Its main properties are presented in table 1. Commercial BDE-209 which is applied to the materials is a mixture that contains 0.3 – 3 % of nonaBDEs and 97 – 99 % of decaBDE (EPA[b] 2006).

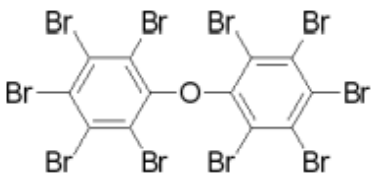


Figure 4 – Structure of the BDE-209

Table 1 – Main properties of the decaBDE
(EHC162 1994; Morf *et al.* 2003)

Chemical formula	**$C_{12}Br_{10}O$**
Relative molecular mass	959.22
Bromine content	81-83 %
Vapour pressure	$< 10^{-6}$ mmHg (20 ºC)
Solubility in water	20-30 µg/L (25 ºC)
log k_{ow}	5.24
Melting point	290-306 ºC
Density	3.0; 3.25
Physical aspect and color	Solid (powder), white
Synonyms	BDE-209, decaBDE, decabromodiphenyl ether, bis-pentabromophenyl, ether pentabromophenyl, decabromodiphenyl oxide, benzene 1, 1' – oxy-bis (2,3,4,5,6 – pentabrominated)
Commercial name	DE-83, DE-83R, Saytex 102E, Adine 505 (e.g.s)

Due to its low vapour pressure and solubility in water, it has been usually assumed that BDE-209 does not present great potential for long-range transport. However, there is recent evidence of its presence in remote sites such as Canadian Lakes, North Pacific Ocean and Arctic (Vonderheide *et al.* 2008; de Wit *et al.* 2010). The presence of this compound in remote sites is related to regional air mass movements. The long-range atmospheric transport seems to play an important role in PBDE movement and distribution at the regional and global scale; for instance, one believes that the dominant source of PBDEs observed in "background" waters and soils is the atmospheric deposition (Vonderheide *et al.* 2008). Thus, it is possible to find the BDE-209 in various environmental matrices such as air, water, soil, sediments. The presence of this compound in environmental matrices results primarily from the manufacture, use and disposal of products in which this compound is applied (Pohl *et al.* 2004; Vonderheide 2009). Due to its properties, BDE-209 tends to accumulate in sediments and soils and is transported preferentially adsorbed to particulate matter. Its high partition coefficients octanol/water and octanol-air (K_{OA}) are related to this high degree of bromination and low water solubility (Vonderheide *et al.* 2008).

According to the literature, decaBDE is a persistent compound in the environment, but this is one of the main requirements for its function when added to a product: the

deceleration of the fire and security against the same over long periods of time, extending to all the life time of the product (BSEF[b]).

Usage and legislation

PBDEs are additive flame-retardants (Alaee *et al.* 2003) which are replacing other flame-retardants currently banned: PCBs (Polychlorinated biphenyls) and PBBs (Polybrominated biphenyls) (Vonderheide 2009).

The most common formulations have many different applications. The pentaBDE was predominantly used as an additive in the furniture industry, especially in polyurethane foams (FPUF); octaBDE was applied to plastics used in several types of equipment, especially ABS resins (acrylonitrile-butadiene-styrene resins) used in musical instruments, for example, and decaBDE is used to almost any type of polymer, for instance, polycarbonates, polyester resins and polyamides (Alaee *et al.* 2003). All applications of BDE-209 can be found in the fact sheet of the Bromine Science and Environmental Forum (BSEF[C] 2006).

As already mentioned above, penta, octa and decaBDE have been the BFRs with largest commercial use and, as such, they have been subjected over the last years to various legal restrictions. In the European Union, the formulations pentaBDE and octaBDE are already banned since August 15, 2004 by the Directive 76/769/EEC (BSEF[a]). RoHS Directive (EBFRIP[a]) governs restrictions on the use of decaBDE. This document bans the use of this compound in the new electrical and electronic equipment from July 1st, 2008 (EBFRIP[a]). Currently, decaBDE is already considered a priority contaminant (Munoz *et al.* 2009) and efforts have been developed to reduce potential emissions: between 2008 and 2009 a reduction of about 60% (from 3,432 Kg to 1.220 Kg per year) was achieved (EBFRIP[b]).

However, it should be noted that the use of decaBDE is only banned for new electrical and electronic equipment. In addition, this prohibition applies only when there are viable alternatives to the use of this flame retardant and when security conditions relating to this alternative are provided (EuropeanUnion 2005). For all these reasons, it is estimated that this contaminant will remain in the environment for several years.

Mechanism of action

Fire is a state, process, or instance of combustion in which fuel or other material is ignited and combined with oxygen giving off light, heat and flame (NWCG 2011). This process is well described by fire triangle presented in figure 5.

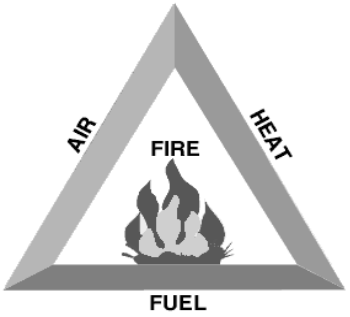


Figure 5 – The fire triangle (BSEF&EBFRIP 2008)

As mentioned above, there are real advantages of incorporation of PBDE in materials, with respect to inflammation and propagation of fire. In the gas phase of a fire, the bromine atoms of these compounds react with free radicals generated during combustion and which are responsible for the spread of fire. Achieved this neutralization of the action of the radicals, the reaction ends (Vonderheide 2009). However, these compounds deserve particular attention and care in handling and application in materials. By not providing stable chemical bonds with the host material, these compounds considered as additives can be more easily released from the materials and / or equipment during use and disposal. This may cause a prolonged and continuous exposure that will have unwanted effects on humans and on the environment.

Distribution in the environment

As noted above and according to what would be expected based on the properties of BDE-209, the concentration of this compound in water is very low relatively to all the content in environmental matrices. The abundance of this compound is distributed by sediments and soils with relative percentages of 52.1 and 46.0 %, respectively, in water is only 1.8 % and in the air this percentage is even lower (0.1 %) (Morf *et al.* 2003).

A review of scientific literature in this area allows us to have a better understanding of the levels of decaBDE found in water. Table 2 presents some results of this review.

Table 2 – Levels of BDE-209 found in water

Type of water	Concentrations (ng/L)	Methods of analysis	References
Wastewater effluents (Norway)	430 – 900	GC – MS	Munoz *et al.* (2009)
Wastewater effluents (Norway)	200 – 600	GC – MS	Munoz *et al.* (2009)
River water (Pearl River Estuarine, China)	0.00381 – 0.00742	GC – MS – NCI	Guan *et al.* (2009)
Guzelyali Port (Turkey)	0.089 ± 0.052 (in summer) 0.054 ± 0.046 (in winter)	GC – MS - ECNI	Cetin and Odabasi (2007)
Prédecelle River (France)	2.100 – 3.810	GC – MS – NCI	Labadie *et al.* (2010)

However, despite these low levels found in water it is important the study of BDE-209 in this environmental matrix because this compound has characteristics of bioaccumulation; moreover, there are already several studies that refer significant levels of BDE-209 in food with an aquatic origin, for instance fish or freshwater birds (Stapleton *et al.* 2004; Elliott *et al.* 2005).

Pathways of exposition and toxicity

There are different sources of human exposure: intake by diet, inhalation of dust and by skin contact. Contact with the skin is the most significant consequence of occupational exposure situations. Workers who carry out functions in local incineration, recycling stations, production and / or disposal of appliances and electronic equipment, for example, are exposed to large amounts of these compounds (Thuresson *et al.* 2006), which are incorporated into the equipment. In these specific situations it is imperative an adequate protection because it is estimated that the exposure of workers is 50 to 200 times (in ng/g lipid weight) higher than that of a worker with other functions that do not involve this type of direct exposure (Vonderheide 2009).

For the general population, the main source of exposure is the food chain. Due to the physical and chemical properties of BDE-209 as indicated above, the foods most likely to transport this compound through the food chain are those with higher fat content. Examples of such foods are meat and dairy products (Eggesbo *et al.*) and fatty fish (waterway) (IRIS-EPA ; ATSDR 2004; Vonderheide 2009). The inhalation exposure (air

samples) is not very common for BDE-209. In contrast, it is more common to find less brominated PBDEs in the air (ATSDR 2004).

Because of the features presented, PBDEs can reach the human body through the lungs (inhalation), stomach (food chain) and, consequently, can reach the bloodstream. Experiments in mammals indicate that BDE-209 can leave the body within a few days in the faeces or in very small amounts in the urine. DecaBDE can be excreted unchanged or as metabolites. On the other hand, the less brominated congeners tend to stay in the human body for years, mainly stored in body fat (IRIS-EPA ; ATSDR 2004).

Under the toxicological point of view, decaBDE presents neurobehavioral effects (NOAEL: 2.22 mg/kg – Reference Dose (R_fD) for oral exposure) and acts as a thyroid hormone disrupter. Furthermore, it contributes to hepatic effects, immunological and lymphoreticular effects and developmental effects (ATSDR 2004). Carcinogenicity in humans was evaluated in the Integrated Risk Information System program from EPA and the compound is referenced in group C, which means that it is "Possible human carcinogen" (IRIS-EPA). Furthermore, there is a study that highlights the toxicity associated with long-term chronic exposure to decaBDE (He *et al.* 2011). In this study, the authors exposed *Zebrafish* (vertebrate model organism) to low doses of BDE-209 (0.001–1 μM or 0.95922 – 959.22 μg/L) from embryonic stage to adulthood continuously for 5 months, in an attempt to simulate the real situation of aquatic animal dwelling in a PBDE contaminated environment. The conclusions raise some concern because the results showed that chronic exposure to low levels of BDE-209 has a significant impact on overall fitness and reproduction (male gamete quantity and quality, gonad development) in parental fish. Moreover, the chronic exposure of *Zebrafish* in aquatic environment to the BDE-209 seems to be associated to delay motor neuron development, loss of muscle fibre and slow locomotion behaviour (He *et al.* 2011).

According to the European Directive 67/548/EEC, hazards classification of BDE-209 is presented on table 3 (LookChem).

Table 3 – Hazards identification

 Warning	Acute toxicity, Inhalation	Category 4
	Acute toxicity, Dermal	Category 4
	Acute toxicity, Oral	Category 4

Precedents and alternatives

As already stated, PBDEs are additive flame-retardants (Alaee *et al.* 2003) which are replacing other flame-retardants currently banned: PCBs (Polychlorinated biphenyls) and PBBs (Polybrominated biphenyls) (Vonderheide 2009).

Nowadays, industry and science are joining to develop sustainable alternatives to the use of halogenated flame-retardants. As has been mentioned too, these compounds are generally toxic, persistent and bio-accumulative, which causes great concern from the point of view of human health and environmental sustainability.

There is already a study recognized by EPA (EPA[c] 2010), entitled "Novel 'Greener' Routes to Halogen-free Flame Retardant Materials" that aims to develop this sustainable alternative. This study (Nagarajan *et al.* 2009) advocates the development of a new class of non-halogenated flame retardant materials based on phenol and substituted phenols. Phenolic derivatives respond to the main requirements for a fire retardant, such as, radical scavenging and char forming capabilities. In this sense, the study proposes the polymerization of substituted phenols, including non-toxic and renewable monomers, such as the cardanol (figure 6). For this polymerization are used 'green' enzymatic (plant peroxidases as catalysts) and biomimetic methods. Note that biomimetic methods relate to the use of living organisms for new functional materials.

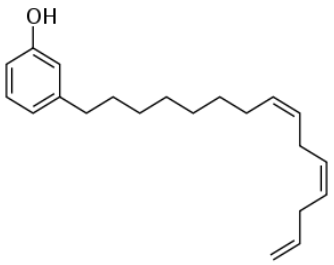


Figure 6 - Cardanol

However, despite the strong points of this promising and environmentally friendly alternative, more studies are needed and their commercial use is not yet applicable.

BDE-209 (photo)degradation

Regarding the phenomenon of degradation in environment, there are different forms of degradation of this type of polybrominated compounds, namely, biological and photochemical degradation (Vonderheide *et al.* 2008). From the experiments performed in the literature (with organic solvents), the by-products of photochemical degradation are less brominated compounds when compared to the parent compound. Less brominated compounds are more toxic than the parent compound (Gandhi *et al.* 2011). Besides the formation of less brominated compounds, there is a possibility of formation of other

compounds such as polybrominated dibenzofurans (PBDFs) (Watanabe and Tatsukawa 1987; Ahn *et al.* 2006; An *et al.* 2008; Christiansson *et al.* 2009).

The study of the photodegradation in water has not been done and it can be important to understand the behaviour of BDE-209 when it is discharged from effluent treatment stations into watercourses. In addition, it is a first step in studies of wastewater treatment through photodegradation, possibly with the use of catalysts. It should also be noted that a recent study showed that BDE-209 can be leach from plastic into bottled water and even there the photodegradation can lead to by-products already mentioned (Andra *et al.* 2012).

In organic solvents, the mechanism of photodegradation of decaBDE is not entirely consensual in the scientific literature, but several authors (Ahn *et al.* 2006; Zeng *et al.* 2008; Christiansson *et al.* 2009; Davis and Stapleton 2009; Shih and Wang 2009; Xie[a] *et al.* 2009) advocate a sequential debromination of the compound by the action of sunlight or ultraviolet radiation. According to the mechanism of reductive debromination, the first step after excitation of the molecule due to the absorption of radiation is the loss of bromine radical by homolytic dissociation (Xie[a] *et al.* 2009). The aryl radical ($Ar^{\cdot}$) formed can receive hydrogen from a hydrogen donor and thus promote the formation of new products (less-brominated compounds) (Suh *et al.* 2009). Xie[a] *et al.* (2009) studied the solvent effects on decaBDE photodegradation and the main conclusions were that a greater ability of solvent to give hydrogen promotes a higher rate of the photolytic process, and that reaction fields formed by dipole/dipole interactions between BDE-209 molecule and different solvents played important roles. However, the same study proposes that the hydrogen addition is not the only way for the photolysis of decaBDE and that other reaction process might occur. The authors of this study performed photolysis experiments of BDE-209 in CCl_4 and they confirmed the BDE-209 photodegradation, although few lower BDEs had been detected (Xie[a] *et al.* 2009).

The mechanism of photodegradation in organic solvents may be repeated sequentially until the formation of tetra-, tri- and dibrominated diphenyl ethers (Zeng *et al.* 2008). Figure 7 shows the most probable sequence of formation of products resulting from the degradation of BDE-209 (Zeng *et al.* 2008; Shih and Wang 2009). This sequence shows the mechanism until the formation of octabrominated compounds by a main reason: the complexity of determining the exact sequence, since different isomers can lead to the

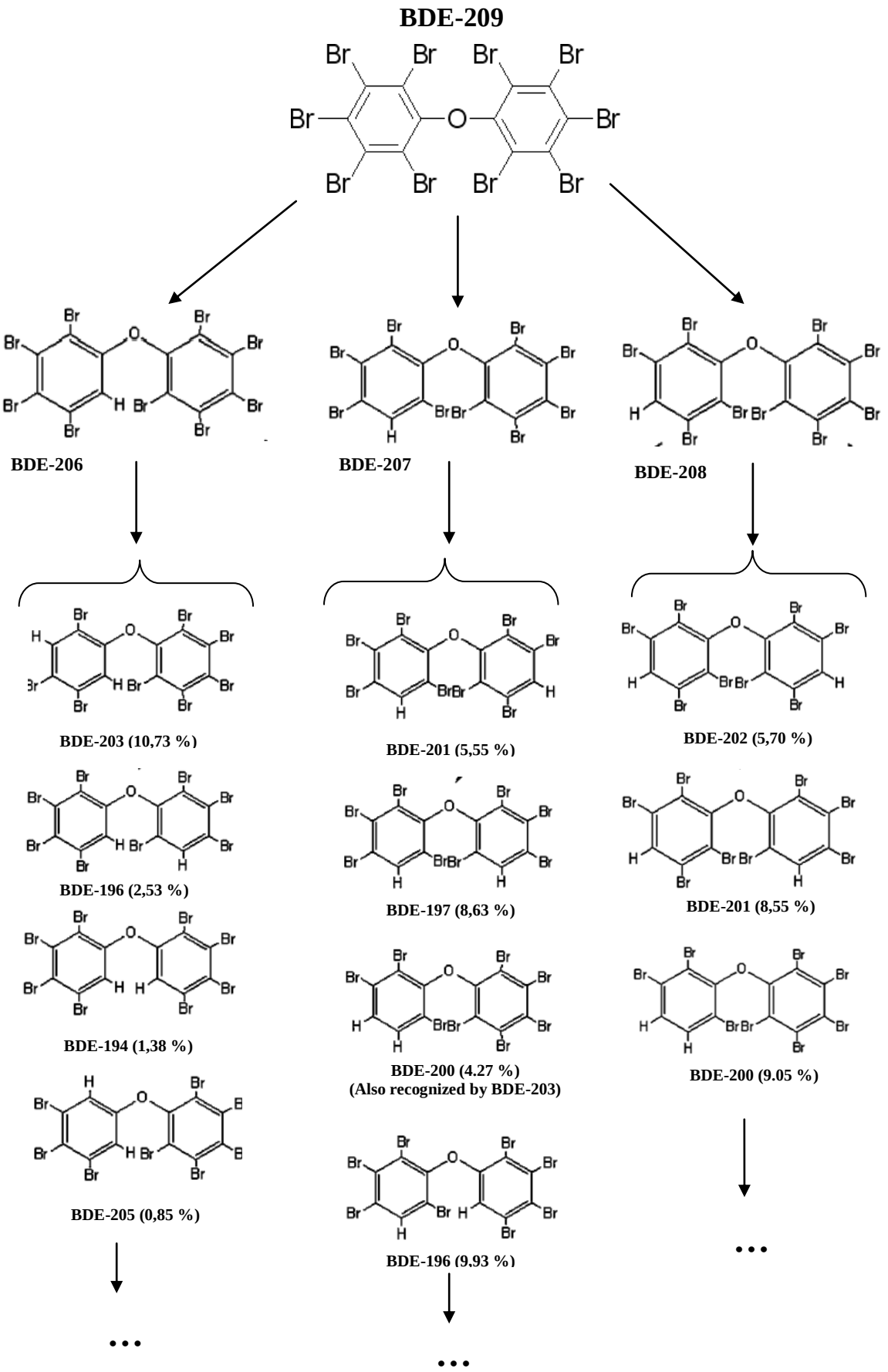


Figure 7 – Main products of the BDE-209 photodegradation (nonaBDE and octaBDE)
(Zeng *et al.* 2008; Shih and Wang 2009)

same photoproducts. It is also important to note that the isomers formed also have a relative order of formation by the action of light. Kinetic studies (Shih and Wang 2009) advocate the following order of formation of isomers: *para* > *meta* > *ortho* position. Therefore, the order of formation of nonabrominated compounds from decaBDE is BDE-208 > BDE-207 > BDE-206.

3. Effect of Humic substances (H.S.) on photodegradation

Humic substances are complex molecules that correspond to a percentage of 50 to 80% of dissolved organic carbon (DOC) in aquatic systems (Thurman 1985). For this reason, it is of great importance the study of the influence of these organic compounds in the photodegradation of BDE-209.

Surface waters are inevitably subject to the action of sunlight. The share of solar energy that reaches aquatic systems is absorbed by dissolved organic carbon through molecular structures called chromophores. The degradation of chromophores (photo-bleaching) arises as a result of stabilization energy by the molecules on which the light falls (Farjalla *et al.* 2009). The absorption of the radiation by the organic matter in aquatic systems induces the molecules to the excited state (Aguer *et al.* 1999) according to reaction 1:

$$HS \xrightarrow{h\nu} {}^{1}SH^{*} \longrightarrow {}^{3}SH^{*} \quad \text{(reaction 1)}$$

Once excited, the humic substances are capable of triggering a series of reactions with the most different species resulting in different types of photo-oxidant species (Aguer *et al.* 1999).

It is possible to distinguish two pathways by which a photosensitizer triplet state ($^{3}HS^{*}$) reacts. On one hand $^{3}HS^{*}$ reacts firstly with the substrate to form free radicals (type I). This pathway involves electron or hydrogen atom transfer reactions between the excited state of the photosensitizer and a substrate, producing radical forms such as hydroxyl radicals (Bancirova 2011; Tavares *et al.* 2011). On the other hand, $^{3}HS^{*}$ may react firstly with molecular oxygen (type II). This type of sensitization process involves energy transfer and produce single oxygen, according to reaction 2 (Li[a] *et al.* 2010; Bancirova 2011; Tavares *et al.* 2011).

$$^3HS^* + {}^3O_2 \rightarrow {}^1HS^* + {}^1O_2 \quad \text{(reaction 2)}$$

Studies about the photodegradation of pollutants in aquatic systems demonstrate that the humic substances influence the persistence time of the compounds in these systems (Okamura and Sugiyama 2004). On one hand, it is known that the humic substances may be responsible for the photo-induced degradation of water pollutants (Aguer *et al.* 1999; Guerard *et al.* 2009) but, on the other hand, they can inhibit the photodegradation of contaminants (Canonica and Laubscher 2008). This inhibition is explained by the competitive absorption for the available photons and by the hydrophobic interaction between pollutants and dissolved organic matter in solution (Bachman and Patterson 1999). Another study suggests that the aromatic compounds bind to the humic substances forming complexes (Rav-Acha and Rebhun 1992). These complexes are considered non-reactive under photodegradative point of view.

The present work presents the study of the effect of three fractions of HS because there are structural differences between them, namely in what concerns elemental composition, aromaticity and functional groups contents (phenolic and carboxylic contents) (Santos 1994; Esteves 1995) and the effects of humic substances on the photodegradation of contaminants depend on their nature (Guerard *et al.* 2009). These fractions of HS are: Humic Acids (HA), Fulvic Acids (FA) and hydrophilic acids (XAD-4). The main properties of these fractions are presented in the figure 8.

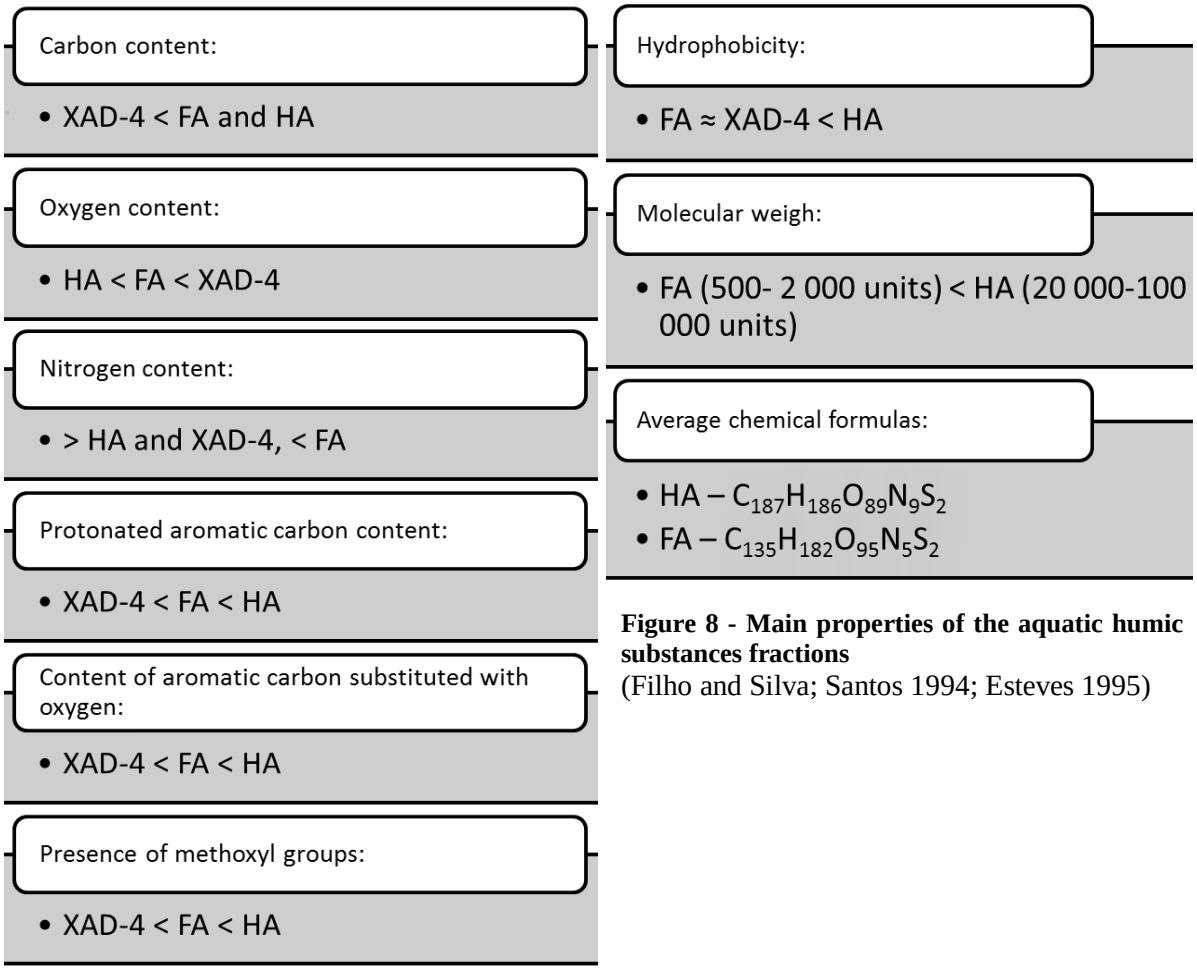


Figure 8 - Main properties of the aquatic humic substances fractions
(Filho and Silva; Santos 1994; Esteves 1995)

4. Sample preparation and analytical methods for photodegradation studies

According to a review of the scientific literature, in the studies of BDE-209 photodegradation in organic solvents, the analytical techniques usually used are GC – MS (gas chromatography coupled with mass spectrometry) (Vonderheide 2009) and HPLC – UV (high performance liquid chromatography with UV detector) (Eriksson *et al.* 2004). In the present work, HPLC – UV was selected due to the availability of the equipment in our research laboratory and due to the good results obtained in preliminary tests.

To date, no studies are known on the photodegradation of BDE-209 in aqueous solution, but it is documented that the solvent nature has a marked influence on its photodegradation, as referred in the last topic of the section 2 of the present work. As a

result of low solubility of BDE-209 in water, the LOD obtained with HPLC – UV was not low enough. Therefore, it was necessary to adopt pre-concentration procedures, which allowed the analysis of BDE-209 at levels lower than BDE-209 aqueous solubility and which are environmentally relevant.

There are several methods of pre-concentration, with particular emphasis to liquid-liquid extraction and liquid-solid extraction.

The conventional liquid-liquid extraction is an expensive and not green technique due to the high volumes of organic solvents used. However, in recent years, a boom has occurred (Cruz-Vera *et al.* 2011; Krylov *et al.* 2011; Zgola-Grzeskowiak and Grzeskowiak 2011) in the development of cheap and easy to use liquid-liquid microextraction techniques in order to pre-concentrate the analytes, allowing their detection at very low concentrations without the need of very expensive techniques such as those of table 2 (hyphenated techniques). Thus, in the present work, a methodology for pre-concentration of BDE-209 had to be developed since the sophisticated techniques presented in table 2 were not available.

Dispersive liquid-liquid micro-extraction (DLLME) is a promising method because it is simple, economical, fast and environmentally friendly since low amounts of organic solvents are used (Rezaee[b] *et al.* 2010). The difficulty in automation is the main disadvantage of the method (Krylov *et al.* 2011). This procedure makes use of ternary solvent system – the aqueous sample, the dispersing agent and the extracting solvent (Krylov *et al.* 2011). The aqueous sample with trace amounts of the analyte of interest will be concentrated in a small volume of organic phase (extracting solvent). The dispersing agent is a solvent that must be soluble in the aqueous phase and in the extracting solvent. The choice of extracting solvent is more complex because it should fulfil a combination of four main requirements: the extracting solvent must have a good ability to extract the compound from the aqueous sample; low water solubility; a good chromatographic behaviour and density greater than the aqueous solution (Li[c] *et al.* 2008; Jian *et al.* 2009; Rezaee[b] *et al.* 2010; Zgola-Grzeskowiak and Grzeskowiak 2011).

As can be seen in figure 9 there are two main steps in the whole procedure of liquid-liquid micro-extraction (Rezaee[b] *et al.* 2010; Zgola-Grzeskowiak and Grzeskowiak 2011):

1) Injection of an appropriate mixture of extracting and dispersing solvents into aqueous sample, containing the analyte;
2) Centrifugation of cloudy solution.

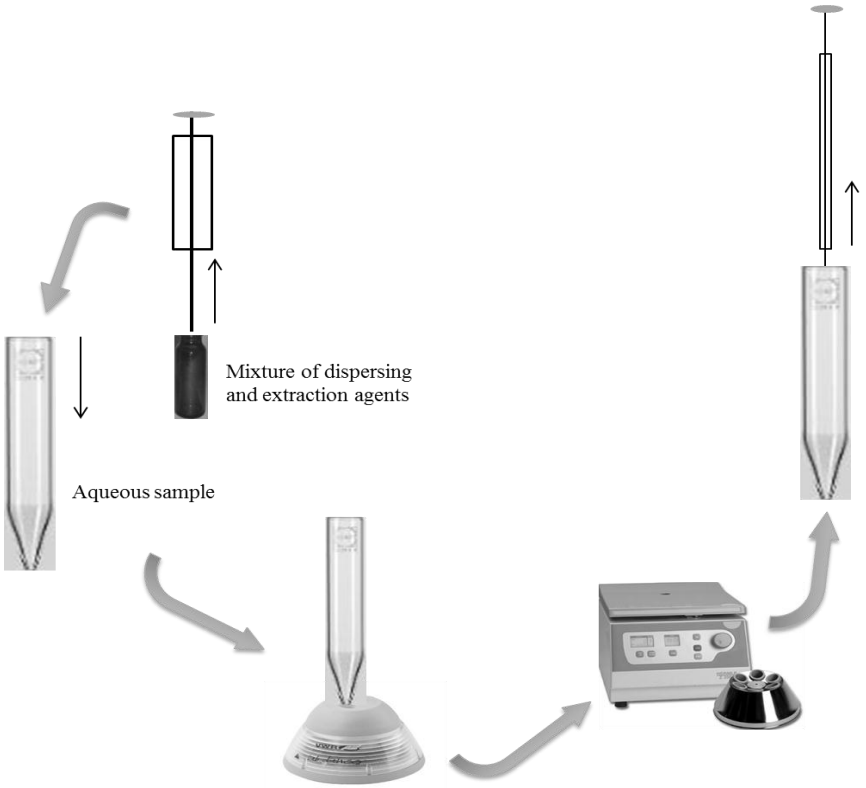


Figure 9 – DLLME procedure, main steps

When the mixture dispersing/extracting solvent is introduced into the sample solution (first step), the extractant is released as a finely dispersed emulsion with the droplet sizes from tenths to hundredths of a micron. This leads to a high surface of contact between extracting solvent and aqueous sample and, as a consequence, the equilibrium is rapidly achieved. In this sense, the extraction process is independent of time (Rezaee[b] *et al.* 2010). The second step allows the sedimentation and separation of the extracting phase for further analysis. This is the reason why the solvent should be denser than water. However, some studies advocate the possibility of using an extracting solvent with a density lower than water. Some of the possible extracting solvents are n-pentane, n-hexane and iso-octane. The main advantage of using this type of solvents is their lower toxicity because, usually, the solvents denser than water are chlorinated hydrocarbons, so they are more toxic solvents (Krylov *et al.* 2011). However, the use of these solvents

requires tubes with a special design to allow the collection of the extracting phase on the top.

Quantitative analysis by HPLC introduces an extra step in the procedure. One of the requirements of the extracting solvent is its good chromatographic behaviour, however most of the solvents commonly used (tetrachloethane, tetrachloroethylene, carbon tetrachloride) (Li[b] *et al.* 2008; Li[c] *et al.* 2008; Jian *et al.* 2009) do not assume this condition. In this study, the extracting solvent used was carbon tetrachloride (CCl_4) and, as can be seen in the chromatogram of the figure 10, CCl_4 absorbs very much at the wavelength of maximum absorption of BDE-209 (230 nm). As it is not completely separated from BDE-209 in the C18 HPLC column used, the sensitivity of analysis method is quite decreased.

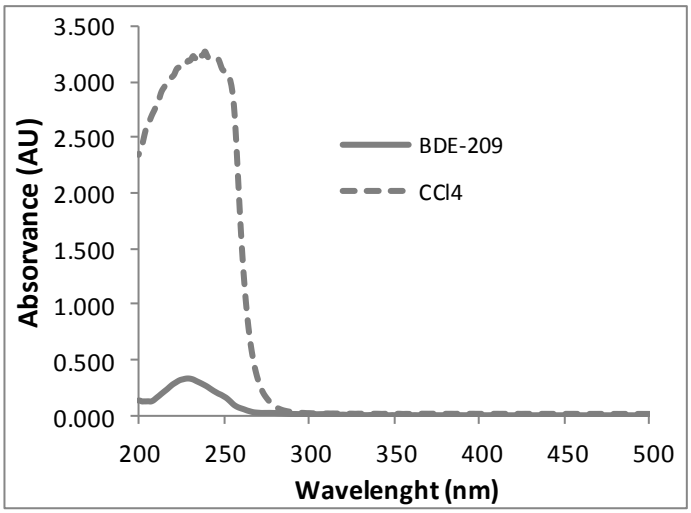


Figure 10 – UV-Vis spectrum of CCl_4 and BDE-209

In the present work, this problem has been solved introducing an additional step which involves the evaporation of the extracting solvent and subsequent recovery of the analyte with acetonitrile.

There are several factors that influence this whole procedure of dispersive micro-extraction (figure 11):

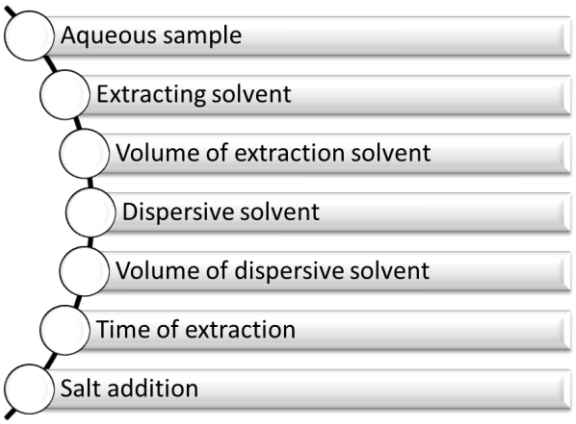


Figure 11 - – Main points of optimization

Among these factors, the proportion between the ternary system solvents – aqueous solution, dispersing agent and extracting solvent – is probably one of the most important points of the whole process. The ideal proportion depends on the compound, the solvents and the method of analysis.

The dispersing and extracting agents features were already mentioned, but should also be highlighted the importance of the volumes used for an optimal extraction. The increase of the volume of extracting agent increases the volume of sedimented phase but the concentration factor is lower and some authors have observed that the percentage of recovery tends to decrease when the added volume is too large (Rezaee[a] *et al.* 2006; Li[c] *et al.* 2008; Jian *et al.* 2009). The recommended volume of extracting agent ranges between 10 and 50 µL, for a volume of the aqueous phase of the order of 5 mL (Krylov *et al.* 2011). With regard to the amount of dispersing agent to use, if we add a small volume, the cloudy state of the solution is not good and the suspension is not stable, as a consequence, the recovery of extraction decreases. On the other hand, if large volumes are added the analyte solubility in the aqueous mixture increases and the extraction efficiency decreases obviously (Rezaee[a] *et al.* 2006). The amount of dispersing agent used is usually 1 mL to a sample volume of 5 mL.

The time of extraction is defined as the time interval between injecting the mixture of dispersing solvent/extracting solvent and starting to centrifugation (Chen *et al.* 2009; Rezaee[b] *et al.* 2010). All experiments described in the scientific literature state that the equilibrium is reached quickly (in seconds) and this is perhaps the main advantage of the procedure DLLME (Krylov *et al.* 2011).

The addition of salt or ionic strength change is a controversial point and is not always easy to explain. There are studies with different hydrophobic compounds that achieve better results by adding salt, often NaCl (Farajzadeh *et al.* 2007; García-López *et al.* 2007; Chen *et al.* 2009; Jian *et al.* 2009) and this can be explained by the salting-out effect. However, there are other studies where the addition of salt does not appear to have any significant effect in improving the results (Farajzadeh *et al.* 2007; Li[b] *et al.* 2008; Li[c] *et al.* 2008).

In quantitative terms, the determination of the pre-concentration factor (PF) (or enrichment factor – EF) can be calculated using equation 1 and the percentage recovery of the extraction (ER) using equations 2 or 3 (Rezaee[a] *et al.* 2006; Rezaee[b] *et al.* 2010).

$$PF = \frac{C_{sed}}{C_0} \quad \text{(eq. 1)}$$

$$ER = \frac{n_{sed}}{n_0} \times 100 = \frac{C_{sed} \times V_{sed}}{C_0 \times V_{aq}} \times 100 \quad \text{(eq. 2)}$$

$$ER = \left(\frac{V_{sed}}{V_{aq}} \right) PF \times 100 \quad \text{(eq. 3)}$$

Preconcentration factor (PF) is defined as the ratio of the analyte concentration in the sedimented phase (C_{sed}) and the initial concentration of analyte (C_0) in the sample. The extraction recovery (ER) is defined as the percentage of the total analyte amount (n_0), extracted to the sedimented phase (n_{sed}). V_{sed} and V_{aq} are the volumes of sedimented phase and sample solution, respectively.

DLLME presents several advantages when it is compared with other pre-concentration methodologies, namely, low cost, low volume of sample, fast extraction of the target analyte and, mainly, the possibility of transport to sampling local. However, as mentioned above, there is another possibility of extraction: the liquid-solid extraction. Solid phase extraction (SPE) is one of the most widely used techniques of liquid-solid extraction. This is a simple procedure and it includes four main steps: 1) preconditioning of the cartridge; 2) addition of the sample; 3) washing and, sometimes, drying of the cartridge; 4) elution of the compound(s) of interest (Almeida *et al.*). The main advantages of this methodology are the higher pre-concentration factor, cleaning of the sample and selectivity. The main disadvantage is the high cost.

Materials and Methods

i. **Chemicals**
ii. **Instrumentation**
iii. **Analytical procedure**

- **Sample preparation:**

Dispersive Liquid-Liquid Microextraction (DLLME) procedure

Solid Phase Extraction (SPE) procedure

- **Photodegradation experiments:**

Photodegradation experiments in aqueous solution

Photodegradation experiments in ethanolic solution

i. Chemicals

BDE-209 (pentabromophenyl ether) (98 %) used in these studies was provided by Sigma Aldrich. For the preparation of solutions, absolute ethanol PA (Panreac) and ultra-pure water obtained from Milli-Q Millipore system (Milli-Q plus 185) were used. Carbon Tetrachloride PA (Panreac) and Acetonitrile, HPLC grade (Lab-Scan, Analytical Sciences) were also used. Methanol (Fisher Scientific) and n-Hexane 95% (Lab-Scan, Analytical Sciences), both HPLC grade, were used for SPE. Commercial Supelclean Envi-18 cartridges (Supelco) of 500 mg, 75 Å pore diameter and 56 μm particle size were set up in a 12-place manifold from Phenomenex to perform the SPE experiments.

To study the influence of humic substances on photodegradation a sample of humic substances isolated from the river Vouga (Carvoeiro, Portugal) during September / October 1991 was used. HS were fractioned into humic acids (HA), fulvic acids (FA) and XAD-4 fraction (also recognized as hydrophilic acids). HS fractions were isolated and their structural characterization was done according to the procedures described by Santos (1994) and Esteves (1995). Table 4 shows results of elemental analysis of HS used in this work.

Table 4 – Elemental composition of the H.S. fractions. Sample isolated in the river Vouga (Carvoeiro, Portugal), September / October 1991 (Santos 1994; Esteves 1995)

	% C	% H	% N	% S	% O
HA	53.4	4.3	3.8	1.2	34.3
FA	54.4	4.9	2.1	0.9	36.4
XAD-4	49.5	4.3	3.2	0.5	40.1

D-Mannitol BioXtra ≥ 98% (Sigma-Aldrich) was used as scavengers of ˙OH.

ii. Instrumentation

Studies of photodegradation under simulated solar radiation were performed using a Solarbox 1500 (Co.fo.me.gra, Italy) equipped with a 1500 W arc xenon lamp and outdoor UV filters.

Quantitative analysis of BDE-209 was achieved by HPLC – UV. This device consists of a degasser DGU-20A5, a bomb LC-20AD, an UV-Vis detector SPD-20A, a column oven CTO-10ASVP (T=25 ºC), all from Shimadzu. A New ACE® C18 column-PFP (5μm), with dimensions of 150 mm x 4.6 mm, and a loop with 20 μL were used. Cell

temperature was maintained at 25 ºC. The mobile phase was 100% acetonitrile and the flow rate was 1.000 mL / min. The detection was done at 230 nm wavelength. The UV-Vis spectra were achieved by UV-2101PC, Shimadzu.

Centrifugation was made using Mixtasel centrifuge, class 2.0 (J. P. Selecta, s.a., Spain). Rotary evaporator (RE111, Buchi, Switzerland) equipped with Water Bath (Buchi 461) was used to evaporate organic solvents.

UV-Vis spectra were obtained with a T90 + UV/VIS Spectrometer (PG Instruments Ltd.).

iii. Analytical procedure

- **Sample preparation:**

Dispersive Liquid-Liquid Microextraction (DLLME) procedure

Due to the low solubility of the compound under study in water, this method had the purpose to pre-concentrate low concentrations of BDE-209 in aqueous solutions. The dispersing agent chosen for this procedure was acetonitrile (most used). For the extracting solvent, we chose carbon tetrachloride (CCl_4) instead of tetrachloroethane (most used), since it is less toxic (MSDS 2005).

DLLME procedure performed in this study (Leal[a] *et al.* 2012), described below, is an adaptation of the procedure described by Li[b] *et al.* (2008). For each experiment three to five replicates were performed.

i. 8.0 mL of the aqueous sample containing 5 µg/L of BDE-209 were placed into 12 mL glass test tube with conical bottom;
ii. the mixture of 1.6 mL CH_3CN (as dispersive solvent) and 45 µL CCL_4 (as extracting solvent) was rapidly injected into the aqueous sample with a 3.00 mL syringe – a cloudy dispersion was formed in the tube;
iii. the suspension was vortexed;
iv. the tubes were covered with Parafilm®, centrifuged during 10 minutes at 4000 rpm and placed on ice during five minutes for a better separation of phases;
v. the fine droplets sedimented at the bottom of tube forming a larger drop which was removed (together with a little amount of aqueous phase) with a 50 µL

micro-syringe (a fixed volume of the 40 ± 1 µL was removed and transferred to small dark vials of 2 mL);

vi. this solution was evaporated to dryness with a low nitrogen flow for about 20 minutes;

vii. the residue of BDE-209 was dissolved in 30 µL of acetonitrile, vortexed and the resulting solution was analysed by HPLC – UV.

The effect of salt addition on DLLME was also studied. For that, 500 µL or 1000 µL of NaCl 17% were added to the tubes after the step ii and before the step iii. The final concentration of NaCl in the sample was 0.9% or 1.9 %, values within the range of experiments referred in the literature (Li[b] *et al.* 2008; Jian *et al.* 2009).

Solid Phase Extraction (SPE) procedure

The procedure used is adapted from others used for PBDEs in different matrices (Covaci and Voorspoels 2005; Medina *et al.* 2009; Nácher-Mestre *et al.* 2010) and consisted of the following steps (three replicates were made for each sample):

I. Conditioning with 6 ml of n-hexane, drying under a stream of air for 30 minutes followed by 6 ml of methanol and 6 mL of Milli-Q water; after drying the cartridge under a stream of air for 30 minutes, the conditioning procedure was repeated (in order to avoid bleeding);

II. Passing the sample through the cartridge (V = 90 mL, pressure: - 40 KPa, flow ≈ 3 ml/min) and subsequently drying under a stream of air during 30 minutes;

III. Eluting BDE-209 with 3 mL of n-Hexane (at atmospheric pressure), drying and recovering in 100 µl of acetonitrile (vortex to dissolve).

- **Photodegradation experiments:**

All solutions of BDE-209 were distributed by quartz tubes (2 x 20 cm) and irradiated in a sunlight simulator, using an irradiance of 54 W/m^2 (290 – 400 nm). This irradiance corresponds to 540 W/m^2 in all spectral range. Three replicates of the photodegradation experiments were made in different days together with the respective dark controls, for each irradiation time. The temperature was measured in each experiment.

Photodegradation experiments in aqueous solution

A stock solution of BDE-209 in acetonitrile, with a concentration of 5 mg/L, was prepared and used to prepare a work solution, a solution with a final concentration of 5 µg/L BDE-209, diluting it with Milli-Q water (0.1 % of acetonitrile). The concentration of 5 µg/L was chosen in order to approach the levels that have been detected in aquatic environments. For the kinetic studies, the aqueous solutions were irradiated during 30, 60, 90, 120 and 150 minutes. After irradiation, 8.0 mL of the aqueous sample was placed in the glass test tube for DLLME procedure.

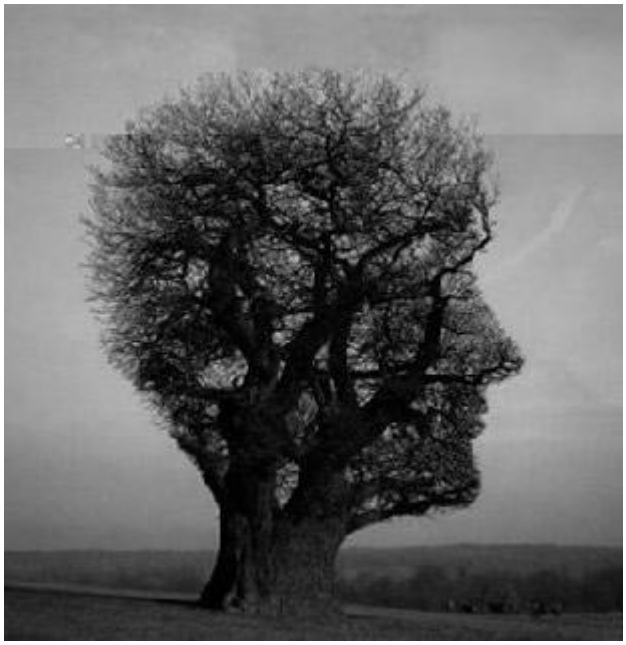
The study of the effect of a specific scavenger on BDE-209 photodegradation was performed in order to evaluate the role of hydroxyl radical (˙OH) on photodegradation of the decaBDE in aqueous solution. For this study, a stock solution of D-Mannitol (˙OH scavenger) was prepared with a concentration of 1 M. From this stock solution, solutions of BDE-209 (5 µg/L) with D-Mannitol (20 mM) were prepared. These solutions were irradiated with the same conditions already described above during 60 minutes. After irradiation, the solutions were subjected to the DLLME procedure and the quantitative analysis was done by HPLC – UV.

For the experiments of BDE-209 photodegradation in aqueous solution, in the presence of HS, stock solutions of each fraction of HS (20 mg/L, approximately) were prepared in water. These solutions were diluted to concentrations very close to those found in unpolluted aquatic environments, about 8 mg/L. The solutions of BDE-209 (5 µg/L) with humic substances were irradiated during 60 minutes. After irradiation, the aqueous samples irradiated were placed into Supelclean Envi-18 cartridges for SPE procedure.

Photodegradation experiments in ethanolic solution

A stock solution of BDE-209 in ethanol was prepared, with a concentration of 5 mg/L. A work solution in ethanol (2.0 mg/L) was placed in quartz tubes and irradiated during 2, 5, 8, 11, 14 and 15 minutes.

For the experiments of BDE-209 photodegradation in ethanolic solution, in the presence of three fractions of HS, stock solutions (16 mg/L, approximately) were prepared in ethanol for each fraction. The concentration of HS in final solution was the same in water and in ethanol (8 mg/L). The irradiation of the solutions of BDE-209 (2 mg/L) with HS, in quartz tubes, were carried out using two irradiation times, 5 and 10 minutes.



Results and Discussion

I. DLLME – HPLC – UV methodology

Identification of experimental details affecting DLLME recoveries obtained

Effect of salt addition

Extraction efficiency

Calibration of the DLLME – HPLC – UV method

II. Photodegradation studies

Kinetics of photodegradation of BDE-209 in aqueous solution

Kinetics of photodegradation of BDE-209 in ethanolic solution

III. Considerations about pathways and photoproducts

Photodegradation pathway and photoproducts in ethanolic solution

Detection of the photoproducts in aqueous solution and the problems of the analysis

Photodegradation pathway in aqueous solution

IV. Influence of Humic Substances on photodegradation

Influence of HS on BDE-209 photodegradation in ethanolic solution

Influence of HS on BDE-209 photodegradation in aqueous solution

I. DLLME – HPLC – UV methodology

Identification of experimental details affecting DLLME recoveries obtained

Despite the simplicity of the dispersive liquid-liquid micro-extraction methodology, throughout the various experiments and analysis, we realized that several experimental details significantly influenced the whole process and, consequently, the efficiency of it.

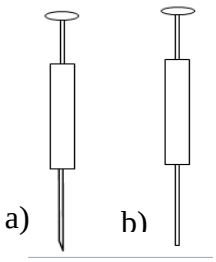


Figure 12 – a) needle with bevel tip; b) needle with shallow tip.

The needle tip of the syringe used to recover the CCl_4 is an important detail on collection of the sedimented phase. Experiments were made with two types of needles: needle with beveled tip and needle with shallow tip (see figure 12) and we found that it was much easier to remove the sedimented phase when a needle with shallow tip was used.

The control of the nitrogen flow during all evaporations was also a critical point for reproducibility of the results. This aspect was addressed, using the support of SPE shown in figure 13, in order to provide simultaneous and well controlled nitrogen streams to evaporate the solvent of six samples in six tubes located inside the glass chamber of the support. This procedure allowed to evaporate more samples at the same time and contributed significantly to increase the precision of the results.

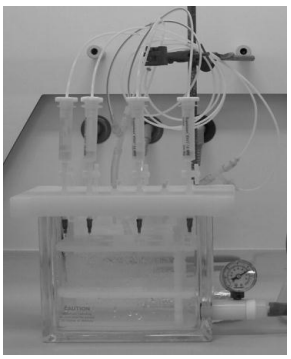


Figure 13 – Adapted system to simultaneous evaporations

After several experiments, it was possible to identify some sources of errors in the preparation of the standard work solutions of BDE-209, as the origin of some variability of the recoveries obtained by DLLME – HPLC – UV.

Since the preparation of standard work solutions for DLLME tests was made by dilution of small volumes (around μL) of the stock solution of BDE-209 in acetonitrile, micropipettes were used for the measurement of the volumes for dilution. However, the tips of the micropipettes have a hydrophobic coating which is not appropriate for measurement of organic solvents and the error in the measurement increases as the volume to be measured decreases. To circumvent this source of error, all amounts of

acetonitrile stock solution used were weighed, and volumes of work solution prepared were always 500 mL.

After suspecting that variations on the time of contact of the aqueous solutions of BDE-209 with the glass container give rise to a considerable decrease in the results precision, two experiments were performed in different occasions, with two independent aqueous solutions of BDE-209. Several analysis of each aqueous solution of BDE-209 (5 µg/L) were performed immediately, 4.5 h and 143 h after preparation of the solution. The treatment of the samples was made by DLLME and the quantitative analysis made by HPLC – UV. The results obtained for the recoveries of BDE-209 are shown in Figure 14.

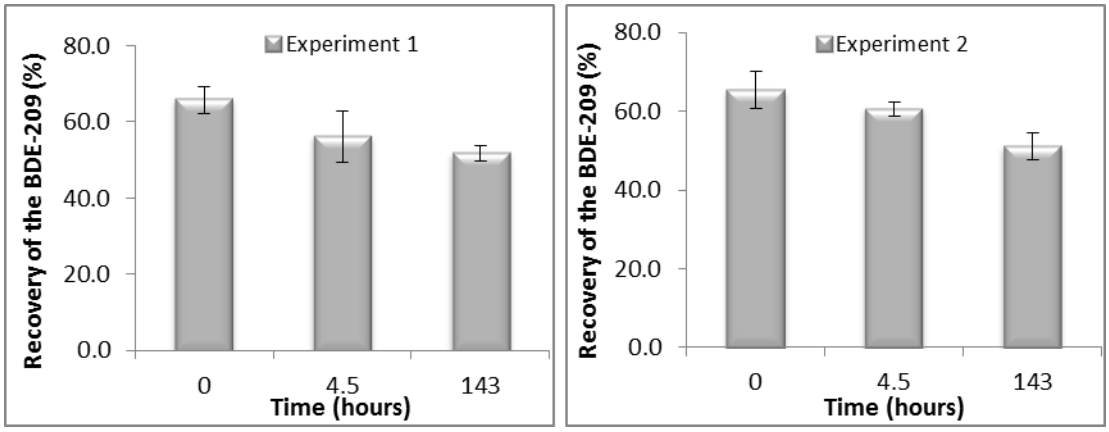


Figure 14 – Adsorption behaviour of the BDE-209 in aqueous solution inside the glass container. Two independent experiments: experiment 1 (left) and experiment 2 (right). Vertical bars correspond to standard deviation of the replicates.

For each solution, a one-way analysis of variance (ANOVA) of the recovery results was performed in order to evaluate if there was a significant difference between recoveries obtained after different times of contact of the solution with the glass container. The statistical analysis of the results (tables 5 and 6) indicates, with a confidence level of 95 %, that there is adsorption of the compound to the flasks along time in both experiments because F is higher than F_{crit} and *p-value* is lower than 0.05. F is calculated by equation 4 and F_{crit} is a tabled value which takes into account the respective degrees of freedom *(Df)*, as well as the level of significance (α). *SS* and *MS* are sum of squares and mean of the sum of squares, respectively. The *p-value* relates to α: if *p-value* is higher than α (α = 0.05, in this case), there are no significant differences. Considering these facts, all solutions should be analyzed immediately after their preparation.

$$F = \frac{\text{between-groups mean square}}{\text{within-groups mean square}} \quad \text{(eq. 4)}$$

Table 5 – ANOVA 1D: adsorption studies of BDE-209 in aqueous solution to the flask – experiment 1

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.47E+08	2	4.73E+08	11.39	0.0017	3.88
Within Groups	4.99E+08	12	4.16E+07			
Total	1.45E+09	14				

Table 6 – ANOVA 1D: adsorption studies of BDE-209 in aqueous solution to the flask – experiment 2

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.65E+08	2	4.83E+08	12.55	0.0011	3.88
Within Groups	4.61E+08	12	3.84E+07			
Total	1.43E+09	14				

Effect of salt addition

Even with low permanence time of the aqueous solution in the flask, the recovery rates were not very high. So, experiments of salt addition were made as described in the section iii of Materials and Methods. The influence of salt addition on recovery of BDE-209 was evaluated by one-way analysis of variance (ANOVA). The statistical analysis of results (table 7) showed that there are no significant differences between the recoveries obtained with the addition of 0, 0.9 and 1.9 % NaCl – value obtained of F is lower than F_{crit}. This is a result that is consistent with other studies already cited, for instance Li[b] et al. (2008). These authors used DLLME to pre-concentrate the BDE-209, using THF (tetrahydrofuran) and tetrachloroethane as dispersing and extracting agents, respectively. In this study (Li[b] et al. 2008), the authors investigated the influence of salt addition (NaCl) – 0 to 3 % (w/v) – and concluded that salt addition had no effect on extraction recovery.

Table 7 – ANOVA 1D: Effect of salt addition

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.16E+08	2	2.08E+08	1.39	0.32	5.14
Within Groups	8.94E+08	6	1.49E+08			
Total	1.31E+09	8				

Extraction efficiency

After the studies above mentioned, the following experimental procedure was used: the methodology DLLME – HPLC – UV was performed as described in experimental part and immediately after of the preparation of the BDE-209 aqueous solution; the amount of stock solution needed to prepare the aqueous solution was weighed; needle with shallow tip was used to remove the sedimented phase; the solvent of the samples was evaporated using the support of SPE to provide simultaneous and well controlled nitrogen streams; the experiments were made without salt addition. For the given conditions, the recovery percentage of BDE-209 achieved for this method was 65.7 ± 3.5 % (n = 10). This recovery was obtained by calibration with standard in acetonitrile (figure 15). The concentrations of BDE-209 standards in acetonitrile ranged between 0.1 and 1.5 mg/L. The value of the coefficient of determination (R^2) was 1.0000 and the detection limit was 0.012 mg/L.

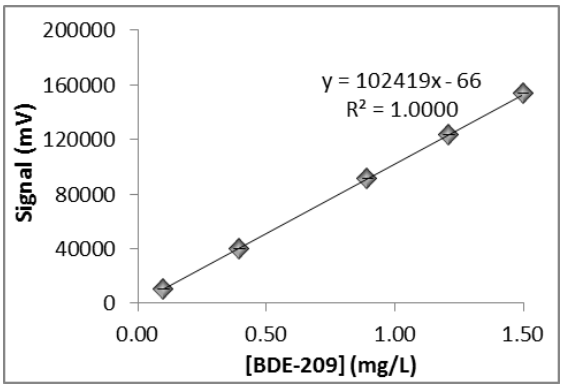


Figure 15 – Calibration curve, in acetonitrile

The real pre-concentration factor obtained (already taking into account the percentage recovery) was about 175 times. Although the value of the percentage recovery obtained is not "ideal," it is a value very similar to results obtained by other authors (69.6 ± 5.2) that used the THF and CCl_4 as dispersing and extracting agents, respectively (Li[c] *et al.* 2008). In addition, it is possible to increase the enrichment factor if several volumes of 40 µL of CCl_4 are removed with a syringe from each tube and are put together in a single bottle for subsequent evaporation and dissolved in 30 µL of acetonitrile.

The small number of papers published that applied the DLLME to pre-concentrate the BDE-209 from aqueous solution before analysis – we found just three (Li[b] *et al.* 2008; Li[c] *et al.* 2008; Jian *et al.* 2009) – use concentrations of BDE-209 in aqueous solution of 100 µg/L while we are conducting studies with a concentration in water of 5 µg/L. The concentration levels that we used are of the same magnitude of the concentrations found in the environment. In addition, the concentration used by these authors is higher than the solubility of BDE-209 presented in Table 1, and can only be explained by the fact that the solutions contained percentages of organic solvent of about 1%, while in the studies that we are developing the solutions contain only 0.1% of organic solvent.

Calibration of the DLLME – HPLC – UV method

As the recoveries are less than 80% and were determined using concentrations of 5 µg/L, we considered important for the study of the BDE-209 photodegradation in water, to verify whether these recoveries remain approximately constant in a range of lower of BDE-209 concentrations up to 5 µg/L. If recovery rates remain approximately constant, the calibration curve obtained with aqueous standards, submitted to the DLLME – HPLC- UV procedure, should have a good correlation coefficient in order to apply the method to aqueous solutions with different concentrations of BDE-209. For this, from a stock solution of BDE-209 (5 mg/L) in acetonitrile five standards of BDE-209 were prepared in aqueous solution (0.1 % acetonitrile) with concentrations ranging between 1 and 5 µg/L. These standards were subjected to the DLLME procedure followed by HPLC – UV analysis. The calibration curve (figure 16), with a coefficient of determination (R^2) of 0.9907, allowed to obtain a detection limit (LOD) of 523 ng/L. The calculation of the limit of detection is done according to equation 5 (Miller and Miller 2000).

$$y_d = a + 3S_{y/x} \qquad \text{(eq. 5)}$$

y_d is detection limit on y-axis, a is intercession with the y-axis and $S_{y/x}$ is the residuals standard deviation of the calibration curve and is calculated using equation 6, where y_i are the experimental y-values for each calibration standard and $\hat{y}_i$ are the calculated values of y using the concentration values of the standards as x_i in the calibration curve

equation; n is the number of standard solutions used for calibration (Miller and Miller 2000).

$$S_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}} \qquad \text{(eq. 6)}$$

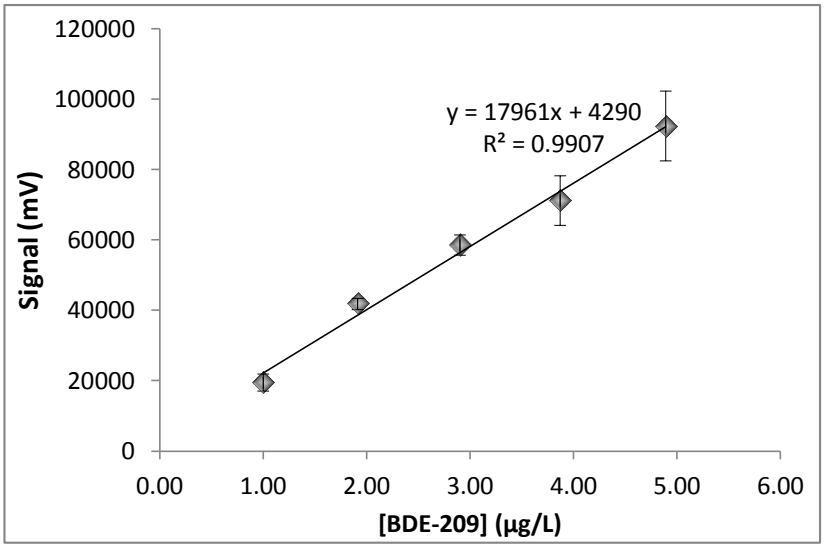


Figure 16 – Calibration curve of DLLME – HPLC – UV method

The calibration curves were not made on every day of analysis, but during the use of a calibration curve, control standards (fresh solutions of BDE-209) were prepared and analyzed on every day of analysis. The peak area of BDE-209 in the chromatograms of these standards was introduced in calibration curve equation to calculate the concentration of BDE-209. The concentrations determined by this procedure did not differ more than 5% compared with the real concentrations.

The results are very promising and offer good starting points for future approaches, namely, for future analysis of aquatic environmental samples. This is a good result which allows to perform photodegradation studies of BDE-209 in aqueous solution with environmental relevant concentrations.

II. Photodegradation studies

Kinetics of photodegradation of BDE-209 in aqueous solution

Three non-simultaneous and independent experiments of BDE-209 photodegradation in aqueous solution were performed with an average irradiance of 540 W/m^2. The temperature was maintained at 35.6 ± 1.6 ºC.

The kinetic results of BDE-209 photodegradation in aqueous solution are summarized in the figure 17, where C_0 and C are the concentrations of the BDE-209 in solutions protected from light or exposed to it, respectively, for different irradiation times. Assuming a pseudo first order kinetics of photodegradation, the experimental data of C/C_0 versus time (t) were fitted by non-linear regression, using GraphPad Prism 5 (demo version) to the following equation (equation 7):

$$C/C_0 = e^{-kt} \qquad \text{(eq.7)},$$

where k is the rate constant, t is time and C_0 and C have the meaning above described. The data for the variation of concentration of BDE-209 along irradiation time were well fitted by the above mentioned equation (R^2 = 0.9687) showing that photodegradation of BDE-209 follows a pseudo first-order kinetics with a rate constant of 0.0101 ± 0.0003 min^{-1}. The half-life ($t_{1/2}$) of BDE-209 was calculated by $t_{1/2}$ = ln(2) / k and the value obtained, in aqueous solution, was 68.4 minutes.

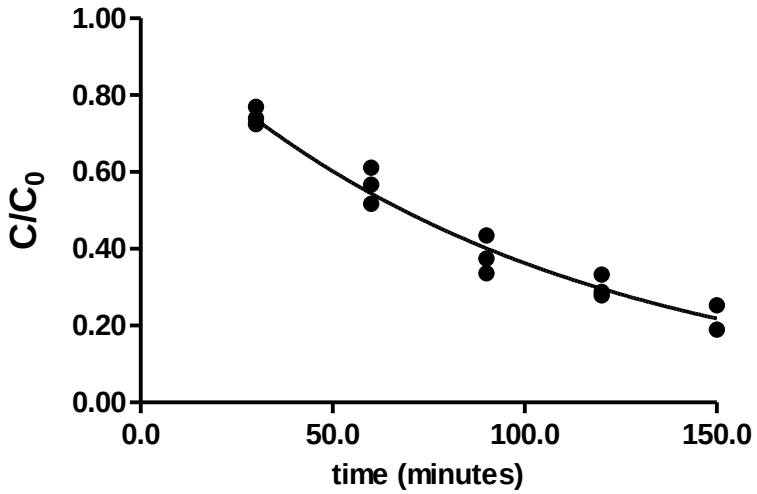


Figure 17 – Kinetics of photodegradation of the BDE-209 in aqueous solution (three replicates) (Leal[b] *et al.* 2012)

Kinetics of photodegradation of BDE-209 in ethanolic solution

For a better comparison between the kinetics of photodegradation in aqueous solution and organic solvents, and since the irradiation conditions are highly variable in the literature, the photodegradation of BDE-209 in ethanol was also studied in the present work, using the same irradiation conditions used for the study of photodegradtion in water.

Ethanol was selected as organic solvent due to its ability to be donor of hydrogen and because it is environmentally friendly when compared to THF (tetrahydrofuran), for instance. According to Eriksson *et al.* (2004) and Xie[a] *et al.* (2009), decaBDE photodegradation is faster in solvents with higher ability to donate hydrogen atoms. For this study, the concentration used was similar to the one used in other published studies and the analysis did not require any pre-treatment of the sample.

Standards of BDE-209 ranging between 0.2 and 2.0 mg/L, with ethanol as solvent, were used to follow the kinetic studies of photodegradation by HPLC – UV. The calibration curves obtained (figure 18, an example) gave coefficients of determination (R^2) ranging between 0.9958 and 0.9993 and limits of detection (LOD) between 0.07 and 0.17 mg/L.

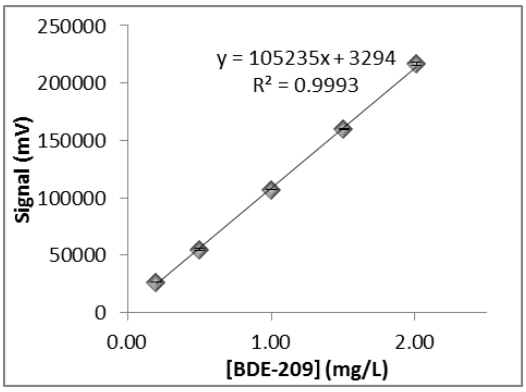


Figure 18 – Calibration curve in ethanol

Figure 19 shows the curve of the kinetics of BDE-209 photodegradation in ethanol and one can observe a rapid photodegradation of the compound. The temperature was maintained at 38.0 ± 1.6 ºC. To the first time of irradiation (2 minutes) is associated a higher variation of results than for bigger times of irradiation due to the difficulty in regulating the value of irradiance in this time. In the figure 19 the curve $C/C_0 = e^{-kt}$, fitted to the data by non-linear regression, is also represented. One more time, the data were well fitted by the mentioned equation (R^2 = 0.9651), what means that the BDE-209 photodegradation in ethanolic solution seems also to follow a pseudo first-order kinetics with a rate constant of 0.208 ± 0.008 min^{-1} and $t_{1/2}$ of BDE-209 photodegradation in ethanolic solution of 3.3 minutes. Comparing the results of both kinetics of photodegradation (figures 17 and 19) one can observe that the BDE-209 photodegradation

is much slower in aqueous solution than in ethanol, with a rate constant about 20 times lower in water than in ethanol.

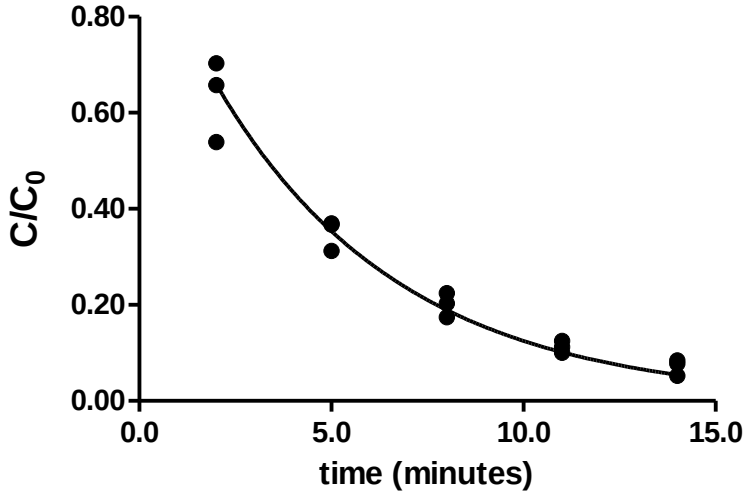


Figure 19 – Kinetics of photodegradation of the BDE-209 in ethanolic solution (three replicates)

The photodegradation rate of the BDE-209 obtained is of the same order of magnitude reported in the literature however the direct comparison is not possible because the experimental conditions are not the same. Xie[a] *et al.* (2009) studied the photodegradation of BDE-209 in ethanol with a 500 W Xe lamp and an irradiance of 3.40 W/m^2 and obtained a rate constant of 0.0197 min^{-1}. Another kinetic study of degradation of the BDE-209 was carried out in hexane (Shih and Wang 2009). In this study, the rate constant obtained with a UV lamp (two 15 W UV lamps) and an irradiance of 714 W/m^2 was 0.073 min^{-1}. In the first study a lower irradiance was used and the photodegradation rate constant is lower than the value obtained in the present work to the photodegradation of BDE-209 in ethanolic solution. In the second study, the irradiance is higher than 540 W/m^2 however it is not necessarily to expect a higher photodegradation rate because the solvent influences the photodegradation rate, and the ability of the hexane in donate hydrogen is lower than that of the ethanol.

In the literature, there is a work (Eriksson *et al.* 2004) that compares the rate constants of the BDE-209 photodegradation in different solvents or mixture of solvents. The authors performed studies of the BDE-209 photodegradation, with UV radiation, in methanol and in methanol:water (80:20) and they concluded that BDE-209 photodegradation in methanol:water was slower than BDE-209 photodegradation in

methanol. So, it seems that there is a tendency that is according to our results because, although the type of radiation is different, the BDE-209 photodegradation in water was slower than BDE-209 photodegradation in ethanol.

III. Considerations about pathways and photoproducts

Photodegradation pathway and photoproducts in ethanolic solution

Figure 20 shows a possible mechanism of decaBDE photodegradation in ethanolic solution. The proposal is based on the mechanism suggested by Suh *et al.* (2009) for the decaBDE photodegradation in tetrahydrofuran (THF). The reaction begins with the absorption of sunlight. As a result, the excited molecule of decaBDE undergoes homolytic cleavage of a C-Br linkage affording two radical species: Ar˙ (specie B) and Br˙. In this step, the solvent plays a strong role because it gives hydrogen for the formation of new products: nonaBDE (specie C) and HBr (step a), figure 20). The fate of the solvent in the radical specie (D) can involve further reactions with oxygen. The removal of hydrogen atom promotes the formation of the hydroperoxide species F. Homolytic cleavage of the peroxide linkage in this specie affords radicals G and H that according to the scheme (step b, figure 20) can originate water and acetic acid.

It is expected that this mechanism of reductive debromination (radical pathway) of the BDE-209 occurs for the formation of nonaBDEs as well as to the formation of other less brominated products. Maybe due to the proximity of the bond dissociation energies (table 8) between O-aryl and aryl-Br, there are authors which defend the possibility of the BDE-209 photodegradation to involve also the cleavage of the O-aryl bond with the generation of phenoxyl and aryl radicals (Rayne *et al.* 2006).

Taking into account the bond dissociations energies presented in the table 8, the order of hydrogen abstraction from ethanol occurs first on – CH_2 –, then – CH_3 and, at last, on – OH.

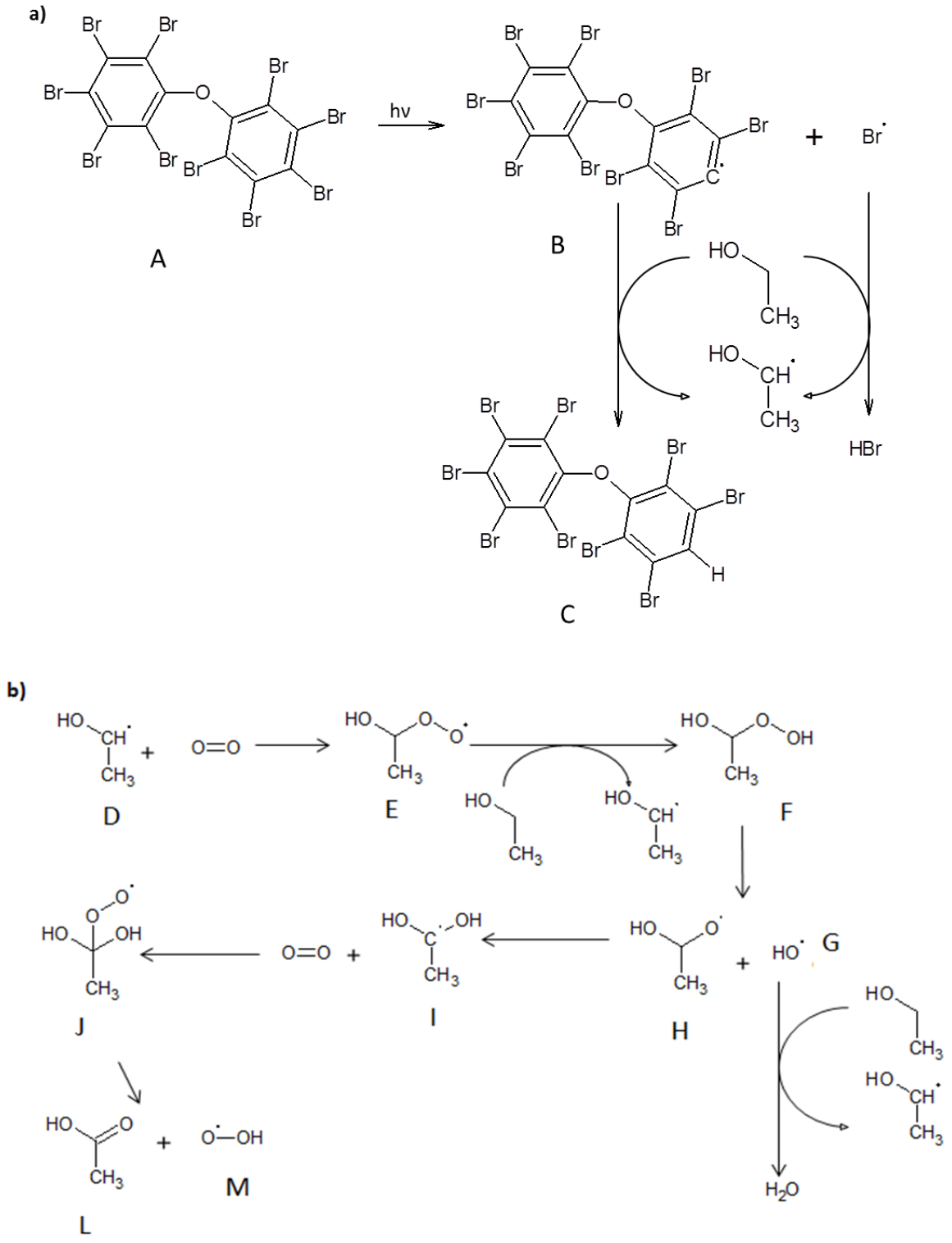
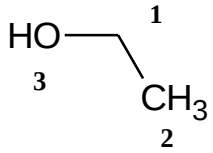
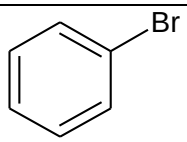
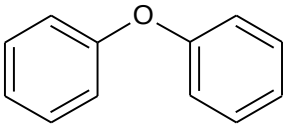
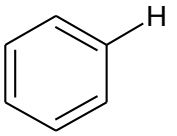


Figure 20 – Proposed photodegradation mechanism of BDE-209, in ethanolic solution.

Table 8 – Bond energies (Luo 2003)

Compounds	ΔH_f^o (Kcal/mol)	Bond dissociation energies (Kcal/mol) (recommended data)
	[1] 13.3 ± 0.8 (C – H) [2] 7.0 (C – H) [3] 3.7 ± 0.8 (O – H)	[1] 94.8 (C – H) [2] 101.3 (C – H) [3] 104.6 ± 0.8 (O – H)
H_2O	9.40 ± 0.05 (O – H)	119.30 ± 0.05 (O – H)
	n.a.	80.4 ± 1.5 (C – Br)
	n.a.	79.4 ± 2 (O – C)
	78.9 ± 0.8 (C – H)	112.9 ± 0.5 (C – H)

n.a. – not available

The chromatograms sequence presented in figure 21 clearly shows the behaviour of the BDE-209 photodegradation. With increasing time of exposure to radiation, the product decomposes and gives rise to other less brominated compounds. According to studies published in the literature (Eriksson *et al.* 2004; Zeng *et al.* 2008; Shih and Wang 2009) less brominated compounds appear at lower retention times than more brominated compounds, this is, the less brominated compounds elute first than more brominated compounds. Comparing the chromatographic profile presented in figure 21 with the profile presented by these authors, the first two peaks that appear just before the compound seems to be nonabrominated compounds. Note that the nonabrominated compounds are three (BDE-208, BDE-207, BDE-206), however based on literature information (Shih and Wang 2009) it can be expected that the first peak at 7 minutes corresponds to BDE-208 and the peak at 6 minutes corresponds to overlapping of BDE-207 and BDE-206.

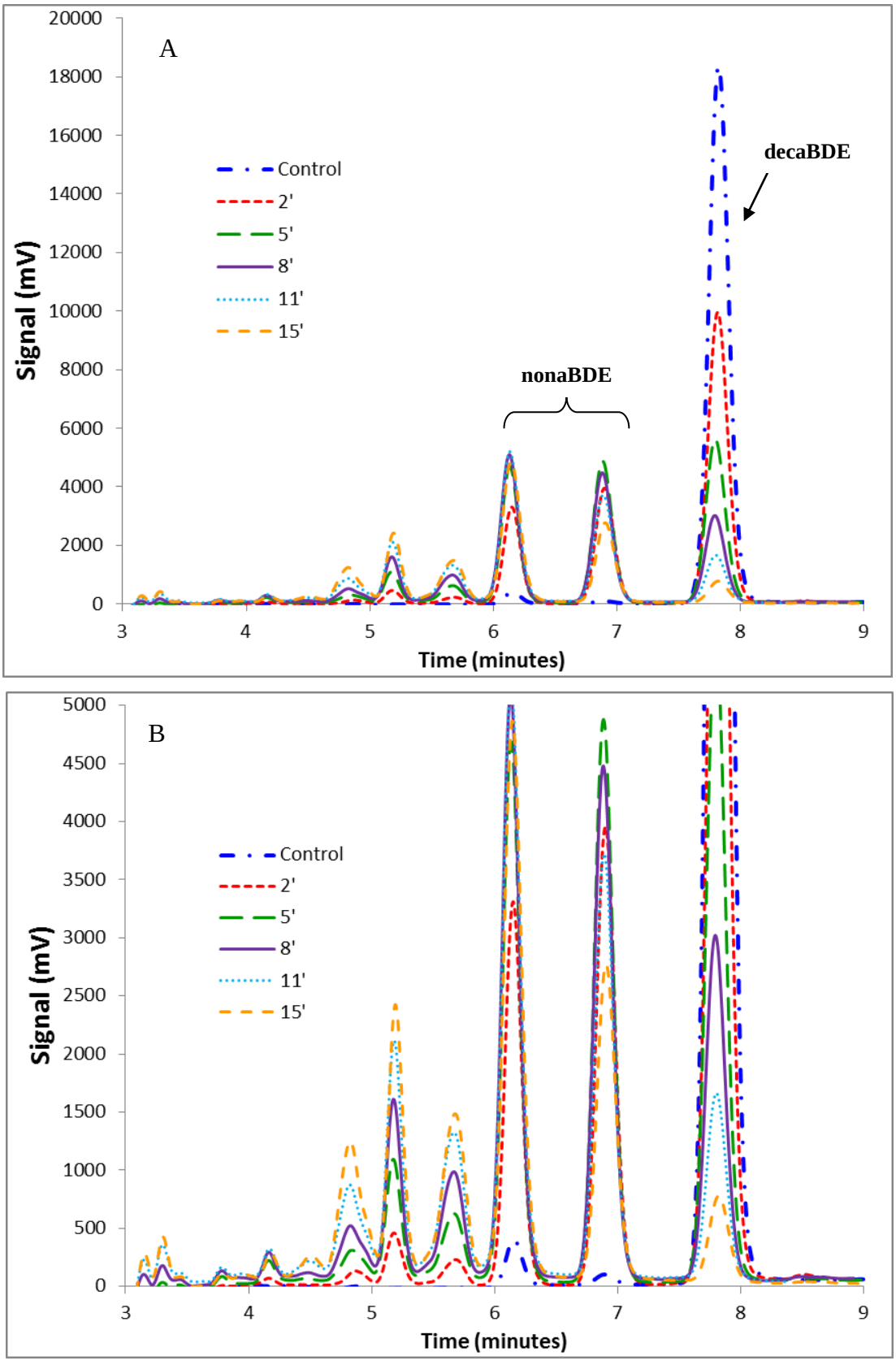


Figure 21 – Chromatograms of the BDE-209 photodegradation in ethanolic solution. Chromatogram B is an enlargement of the chromatogram A.

Although the reductive debromination is the most consensual mechanism of the BDE-209 photodegradation, there are some authors which defend too the formation of polybrominated dibenzofurans (PBDFs) (Bieniek *et al.* 1989; Rahman *et al.* 2001; Eriksson *et al.* 2004; Zeng *et al.* 2008; Christiansson *et al.* 2009). The most consensual hypothesis is that the PBDFs do not form directly from BDE-209 and that PBDFs may be formed by different routes (Eriksson *et al.* 2004; Christiansson *et al.* 2009). Christiansson *et al*. (2009) defend that an excited PBDE undergoes intramolecular reaction to form a PBDF instead of reacting with the surrounding solvent and, furthermore, that PBDEs may also fragment to form phenols. Other authors proposed two possible routes to formation of PBDFs (figure 22), based on behaviour of other organic compounds, the PCDEs (polychlorinated diphenyl ethers) (Lenoir *et al.* 1991; Rahman *et al.* 2001). Another aspect to stand out is that the authors which presented the possibility of PBDFs formation did not achieve to detect the PBDFs during BDE-209 photodegradation, maybe due to high quantum yields, high absorption coefficients (Lenoir *et al.* 1991; Christiansson *et al.* 2009), high decomposition rate and stronger absorbance at longer wavelength of PBDFs (Eriksson *et al.* 2004).

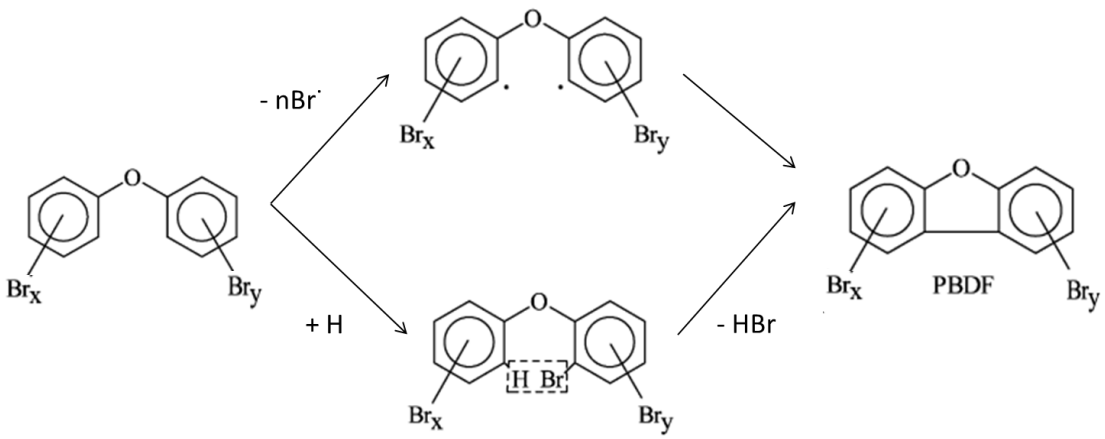


Figure 22 – Two possible routes of formation of PBDFs
(Adapted of Lenoir *et al.* 1991; Rahman *et al.* 2001)

Another aspect that may also justify the formation of PBDFs or maybe others products uncharacterized is mass balance. Eriksson *et al.* (2004) observed a decrease of the total area under the peaks in HPLC chromatogram after most of the BDE-209 had decomposed, what suggest a loss of mass.

It is also important not to forget that there are hydroxyl radicals in medium (figure 20, species G) and that it is possible the formation of CO_2 and H_2O during the photodegradation process, one hypothesis defended also by An *et al.* (2008) who studied the degradation of decaBDE with immobilized TiO_2.

Detection of the photoproducts in aqueous solution and the problems of the analysis

As has been referred during the present work, to our knowledge, this is the first study of the BDE-209 photodegradation in aqueous solution. A previous attempt has been made by Erikson *et al*. (2004) with aqueous solutions containing about 50 μg/L of BDE-209 and 1% ethanol but the authors claimed that the results were rather poor and that studies in aqueous phase were practically impossible. As a consequence, in our work, there were some problems during the analytical procedure to obtain the best approach to the study of the BDE-209 photodegradation and for the detection of photoproducts and its degradation pathway.

The study of the BDE-209 photodegradation in water started by the irradiation of the samples (during five different times) followed by DLLME procedure to concentrate them. The chromatogram A of the figure 23 shows an example of the photodegradation behaviour of two irradiated solution along 60 minutes: one protected from light (60' protected) and the other exposed to it (60' exposed). The chromatograms B and C of this figure are a separate enlargement of each solution (protected or exposed to light). The analysis of these chromatograms proves not only the degradation of decaBDE but also suggests the occurrence of at least three products of degradation – peaks a), b) and c). The peak at 19 minutes, approximately, does not concern any photodegradation product because it is also present in the chromatogram of the control (60' protected).

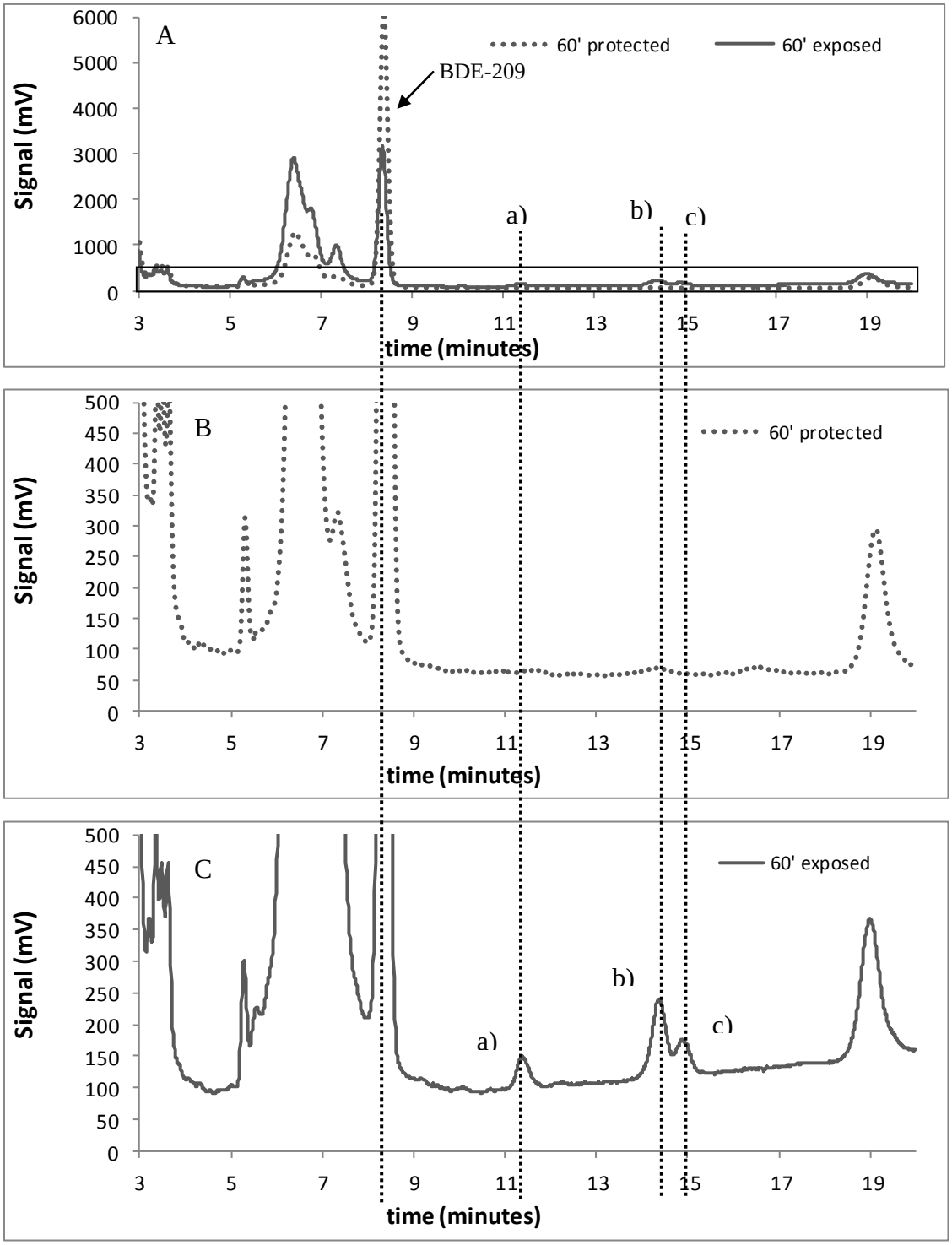


Figure 23 – Chromatograms of irradiated aqueous solutions of BDE-209 after 60' of irradiation: solution protected (- - -) and exposed to light (------). Method of extraction and analysis: DLLME – HPLC – UV. The chromatograms B and C are an enlargement of the chromatogram A. The vertical lines make the correspondence between the same peaks in all chromatograms.

Although the DLLME procedure is a good methodology for the kinetic study of the BDE-209 photodegradation – due to the real advantages which presents relatively to other pre-concentration methodologies – the blank of the analytical procedure, DLLME – HPLC – UV, obtained with Milli-Q water as sample, presented a chromatogram with several peaks/bands. Based on the possible existence of other degradation products that may be hidden by the blank, other attempts of pre-concentration and extraction techniques of the BDE-209 and its photoproducts were made. These preliminary studies rely primarily to the use of the rotary evaporator – technique more accessible – and to the use of solid phase extraction (SPE). In both cases, because they are preliminary experiments, the photodegradation studies were performed to a single irradiation time (60 minutes). The choice of this irradiation time was due not only to its proximity to the half-life of BDE-209 in water ($t_{1/2}$ = 68.4 minutes), but also because this is the time for which the higher number of peaks were clearly visible in the chromatograms of solutions obtained after DLLME treatment of the irradiated samples.

As a first attempt, the irradiated solution of BDE-209 was poured into the round bottom flask of centrifugation; evaporation was carried out under vacuum until dryness and the residue was re-dissolved in a small amount of acetonitrile. The organic solution was then evaporated to reduce the volume to a minimum, enough for injection into HPLC. The chromatogram A of the figure 24 shows an example of the photodegradation behaviour of two irradiated solution along 60 minutes: one protected from light (60' protected) and the other exposed to it (60' exposed). With this methodology (rotary evaporator – HPLC – UV) it was possible identify two new peaks – peak d) and e) – furthermore the peaks already identify by methodology DLLME – HPLC – UV.

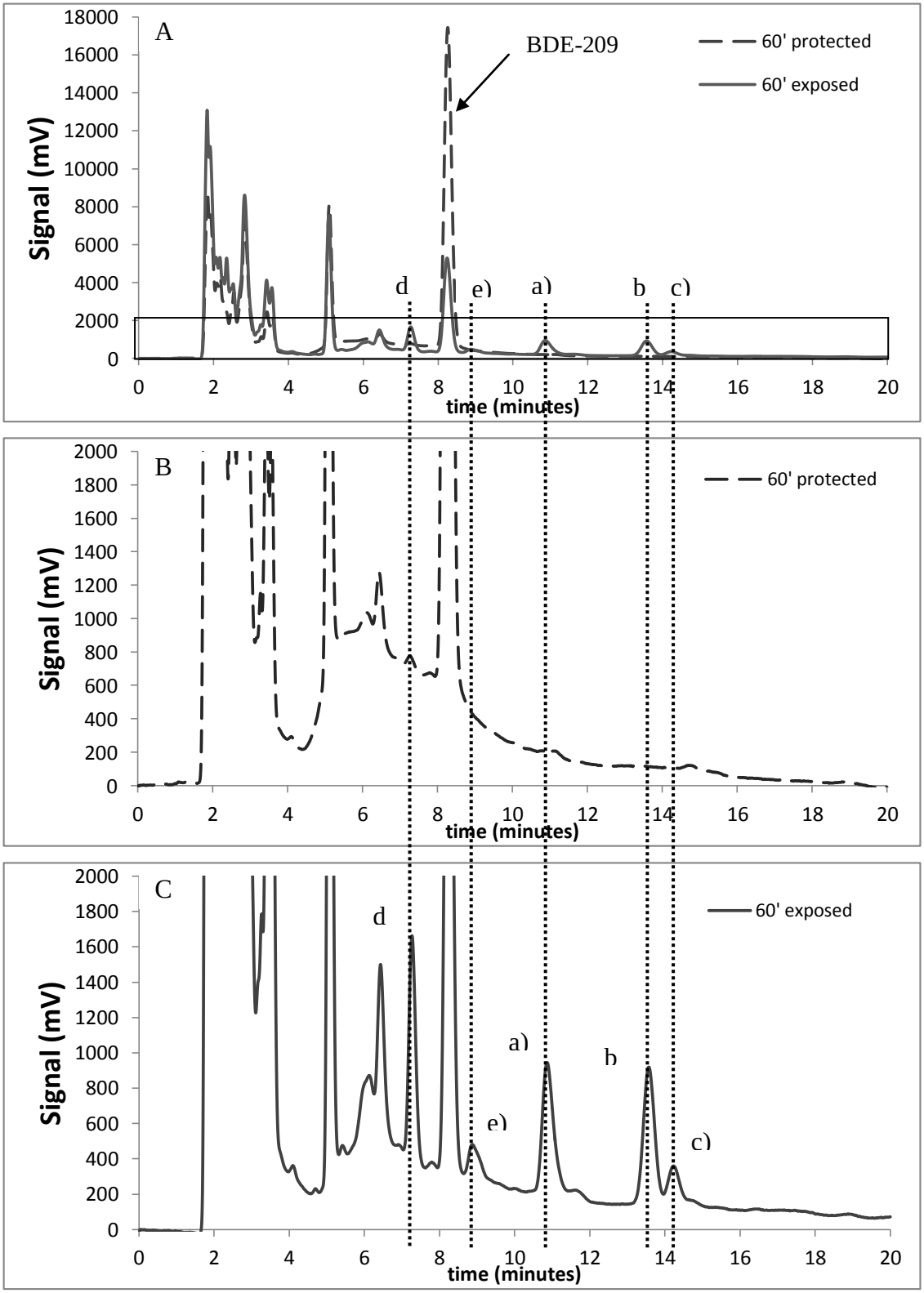


Figure 24 – Chromatograms of irradiated aqueous solutions of BDE-209 after 60' of irradiation: solution protected (- - -) and exposed to light (------). Method of extraction and analysis: rotary evaporator – HPLC – UV. The chromatograms B and C are an enlargement of the chromatogram A. The vertical lines make the correspondence between the same peaks in all chromatograms.

This experiment allowed to detected new peaks (qualitative analysis), but as the procedure was very slow, we considered it was important to try the methodology SPE – HPLC – UV, a more expensive methodology but with more advantages, namely, cleaning of the sample, selectivity and pre-concentration factor . Due to the space limitations in the Solarbox (sunlight simulator) and taking into account the capacity of the quartz tubes (maximum volume of 50 ml) and the experience time that should not be too large (to reduce adsorption), we considered that the maximum volume of aqueous sample that could pass through the SPE cartridge was 90 ml. Since a volume of 3 ml of eluent might not be sufficient to achieve detection of degradation products (concentration factor of about 30 times, and lower than with the DLLME), we decided to make the elution of the decaBDE with 3 ml of eluent followed by evaporation of the eluate to dryness and re-dissolution of the residue in 100 µl of CH_3CN, allowing a concentration factor of about 900 times (assuming a recovery percentage of 100%).

Several solvents have been considered for the elution of BDE-209 and its photoproducts from the SPE cartridges. However, we took into account that the blanks of the DLLME – HPLC – UV procedure exhibited various signals in the chromatograms of the final solutions in acetonitrile, which were not present in standard solutions of BDE-209 in acetonitrile directly injected in the chromatograph. Thus, we decided test first the effect of evaporation and recovery in CH_3CN using standard solutions of BDE-209 in different solvents. Standards of BDE-209 with the same concentration (0.1 mg/L) were prepared in four different organic solvents: acetonitrile, n-Hexane, carbon tetrachloride and tetrahydrofuran. Each standard was then evaporated under a nitrogen stream and the compound dissolved in acetonitrile for injection into HPLC. The results presented in the figure 25 showed that THF cannot be used because the chromatogram presents many signals which can hide the peaks of the photoproducts of BDE-209. Comparing the peak areas of BDE-209 in the four chromatograms one can observe that in the case of the standard in CCl_4 the area is slightly smaller, suggesting a lower recovery. Thus, the better options to detect possible photoproducts peaks in the interval between 4 and 8 minutes are the solvents acetonitrile (chromatogram a), figure 25) and n-hexane (chromatogram b), figure 25).

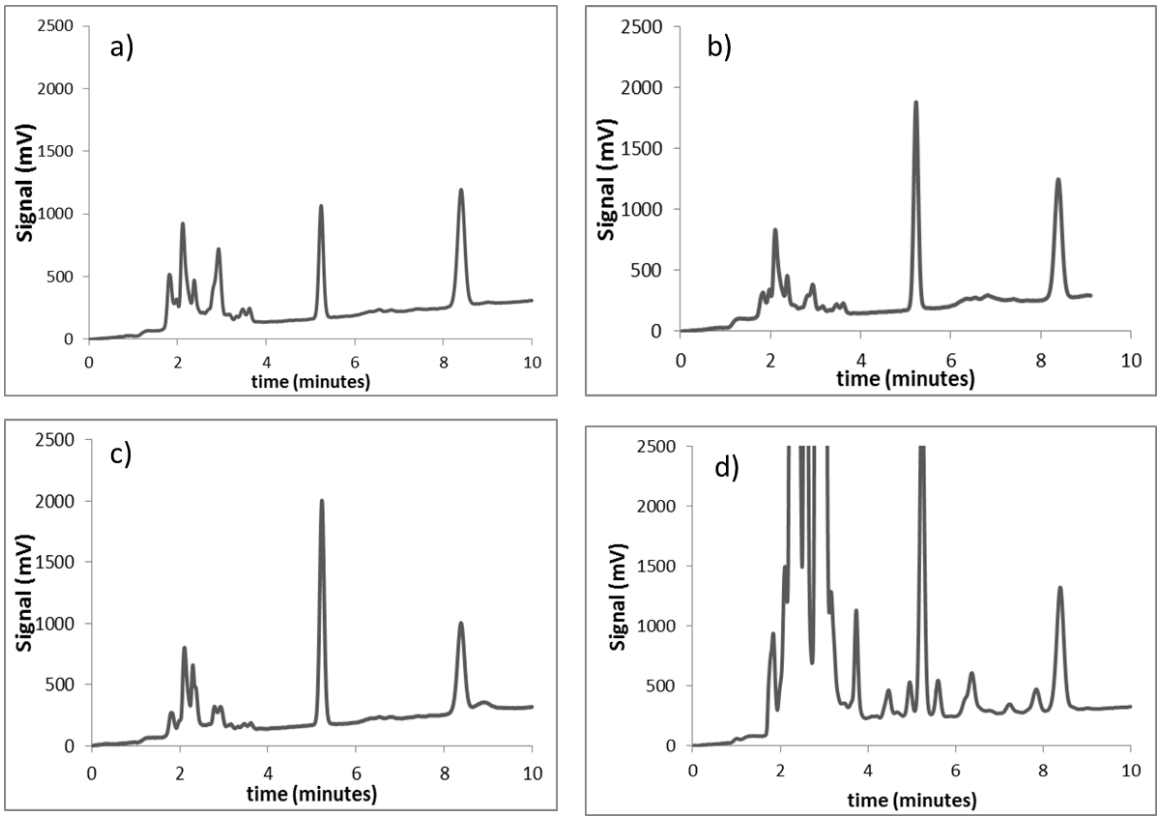


Figure 25 – Standard of BDE-209 (0.1 mg/L) in different organic solvents evaporated under nitrogen stream and re-dissolved in acetonitrile. a) Standard in acetonitrile; b) standard in n-Hexane; c) standard in carbon tetrachloride; d) standard in tetrahydrofuran.

After the analysis of the results of this last experiment, and since even the solution in acetonitrile, after evaporation and re-dissolution, presented additional peaks in the chromatogram which were not present when the solution was injected directly, another test was done related to the possibility of contamination during the evaporation step. Accordingly, we compared two methods of evaporation (with nitrogen or compressed air) of the standard solution in acetonitrile. For that, the same standard of BDE-209 in acetonitrile was analysed following three different procedures:

- the standard solution was directly injected into the chromatograph and analysed by HPLC – UV (chromatogram a, in Figure 26);
- 3 mL of the standard solution were evaporated with a nitrogen stream until dryness and the residue was re-dissolved again in 3 mL of acetonitrile and this solution was analysed (chromatogram b, in Figure 26);

- 3 mL of the standard solution were evaporated with compressed air until dryness and the residue was re-dissolved again in 3 mL of acetonitrile and this solution was analysed (chromatogram c, in Figure 26).

The results presented in figure 26 clearly showed that there is contamination during evaporation steps and that the better way to evaporate, when it is necessary, is with the compressed air because the contamination seems to be smaller than the evaporation with the nitrogen flow.

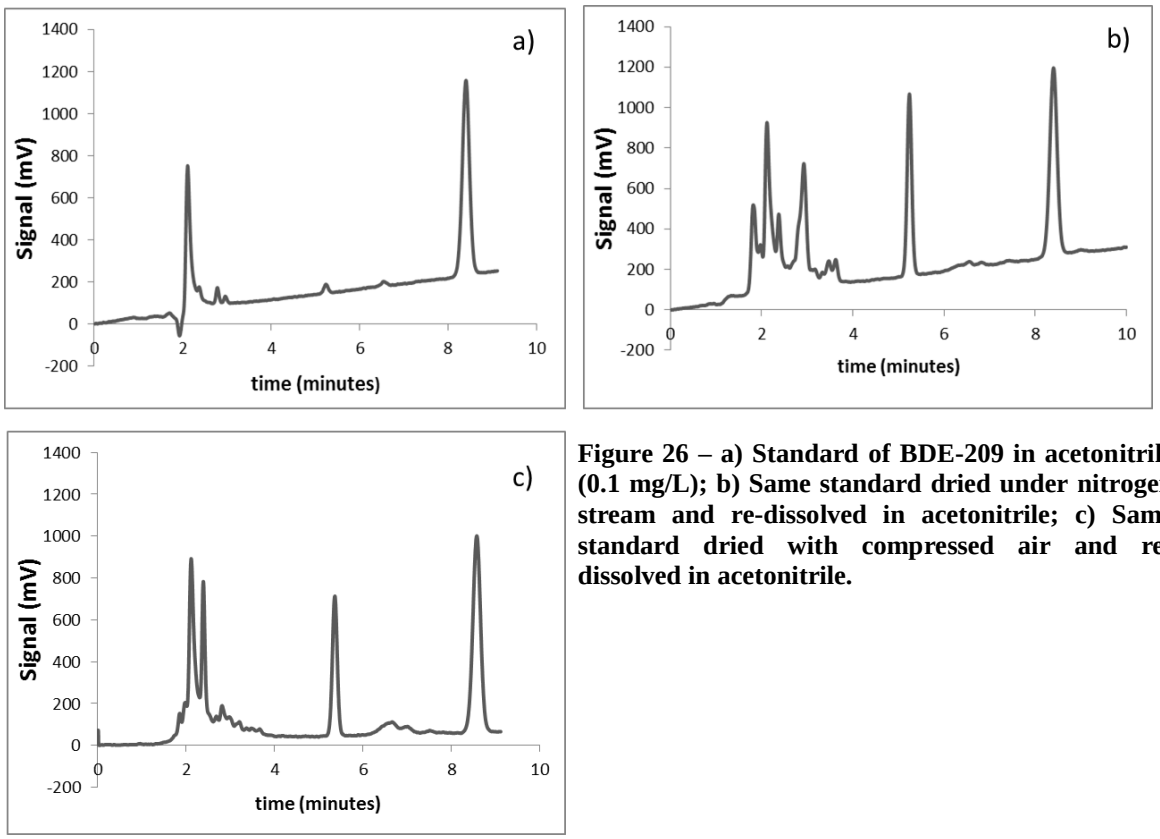


Figure 26 – a) Standard of BDE-209 in acetonitrile (0.1 mg/L); b) Same standard dried under nitrogen stream and re-dissolved in acetonitrile; c) Same standard dried with compressed air and re-dissolved in acetonitrile.

Taking into account the results presented above, the solid phase extraction was performed using the n–hexane as solvent for elution and compressed air for drying the eluate before re-dissolution in acetonitrile. Hexane was chosen because it is less polar than acetonitrile therefore, it has a better ability to elute the decaBDE and, eventually, their less brominated photoproducts which, according to our preliminary study, may also be present. The results achieved by the methodology SPE – HPLC – UV are presented in the figure 27.

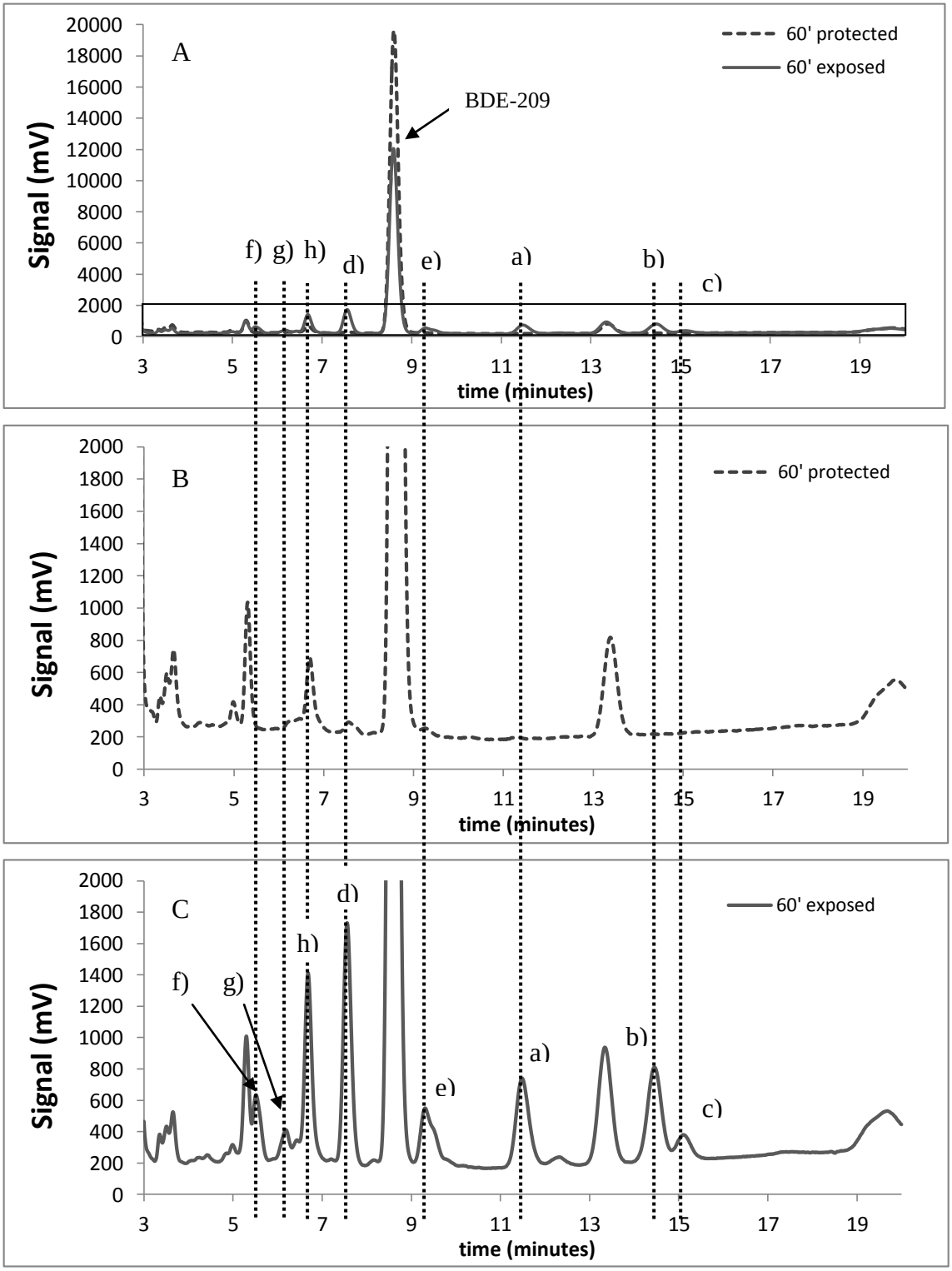


Figure 27 – Chromatograms of the aqueous solutions of BDE-209 after 60 minutes of irradiation: solution protected (- - -) and exposed to light (------). Method of extraction and analysis: SPE– HPLC – UV. The chromatograms B and C are an enlargement of the chromatogram A. The vertical lines make the correspondence between the same peaks in all chromatograms.

Figure 27 shows eight peaks corresponding to products of the photodegradation of decaBDE, three more peaks – f), g) and h) – than the number of the peaks detected using the procedure with the rotary evaporator – HPLC – UV. In the figure 27, the chromatogram A shows an example of the photodegradation behaviour of two irradiated solution along 60 minutes: one protected from light (60' protected) and the other exposed to it (60' exposed). The chromatograms B and C of this figure are a separate enlargement of each solution (protected or exposed to light).

To sum up, three peaks – a), b) and c) – were detected with DLLME procedure; five peaks – a), b), c), d) and e) – were detected with rotary evaporator procedure and eight peaks – a), b), c), d), e), f), g), h) – were detected with SPE procedure. Comparing the chromatograms obtained by the three methodologies, it is possible to conclude that the best methodology for the detection of the products of BDE-209 photodegradation is SPE – HPLC – UV because it allows the detection of a higher number of peaks, presents chromatograms with less impurities (due to the cleaning of the sample) and allows a higher pre-concentration factor.

The compounds eluted after BDE-209 are not known but it is expectable that they are compounds less polar than BDE-209 because the HPLC column is reverse-phase and more polar compounds elute first. On the other hand, the compounds that elute before BDE-209 appear to be lower brominated compounds by comparison of retention times of the peaks in the chromatograms of the irradiated solutions of BDE-209 in ethanol and in water. These results are contradictory with literature and are completely new. Eriksson et *al*. (2004) defined the photolysis experiments of BDE-209 in aqueous solutions as "practically impossible" and did not found photoproducts in similar analytical conditions (HPLC – UV, C18-RP). Hardy (2002) go further and advocate that lower brominated compounds are not formed during decaBDE photodegradation in water, based on the behaviour of other halogenated aromatic compounds.

Photodegradation pathway in aqueous solution

Figure 28 presents some possible routes of formation of the BDE-209 photoproducts. The first step is the same in ethanol and in water: the excitation of the molecule by radiation and the formation of the radical specie **I**. After this step different reactions (A – E) may occur.

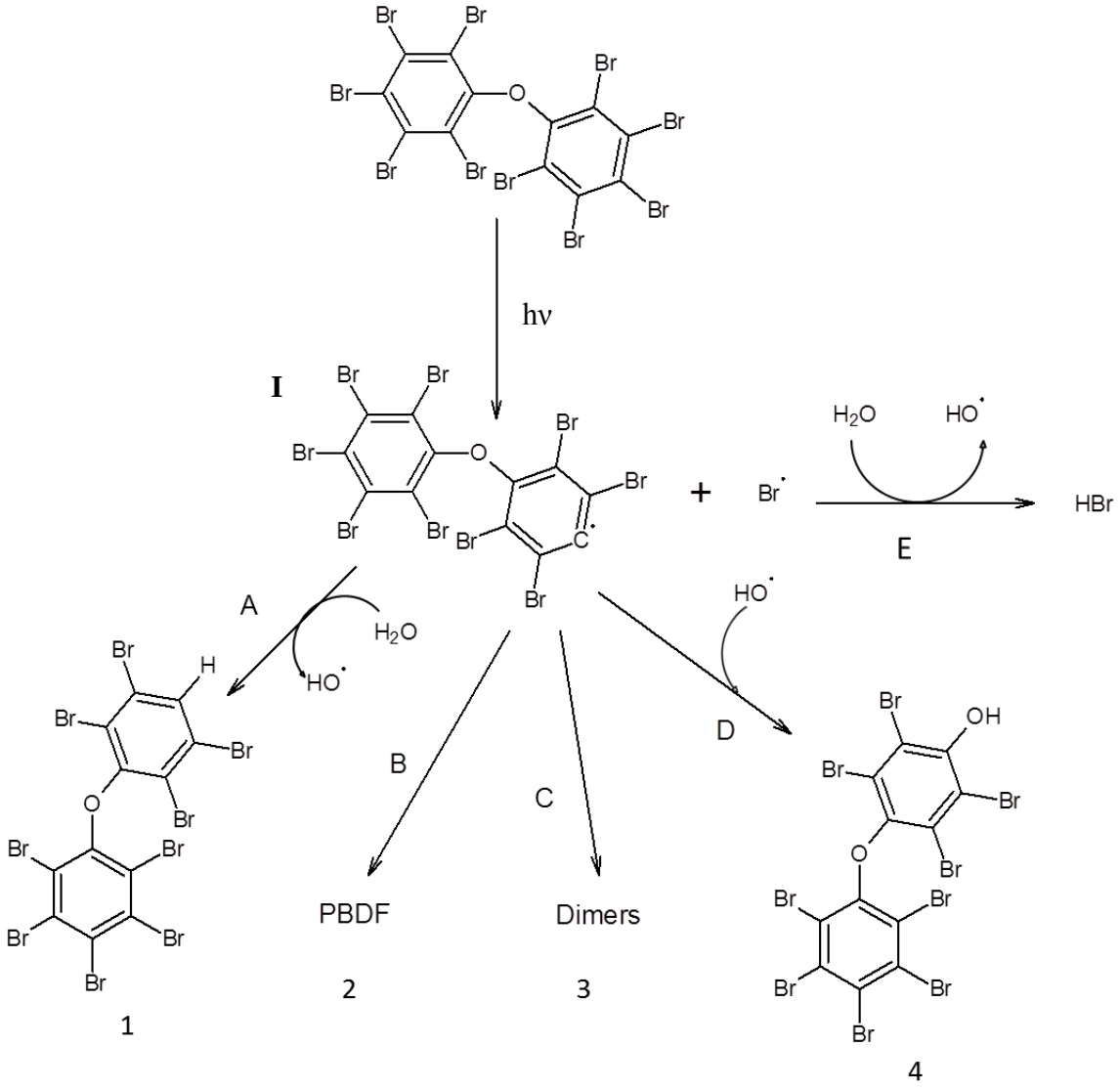


Figure 28– Possible routes of the BDE-209 photodegradation, in aqueous solution

As mentioned above, there are several authors who defend that the formation of lower brominated diphenyl ethers does not occur in water (Hardy 2002; Norris et al. 1974). Taking into account the thermodynamic data in table 8 we assume the possibility of occurrence of the reaction with water (reaction A) as a pathway for the formation of lower BDEs (species 1, figure 28) which presence in irradiated aqueous solution is suggested by the analysis of the chromatograms as discussed above (Figure 27). Hydroxylated species (species 4, figure 28) may also be formed by reaction with hydroxyl radicals (reaction D). Hardy (2002) and Norris *et al.* (1974) considered that an oxidative process of photo-hydroxylation leading to the formation of phenolic compounds was the favoured pathway

of degradation in water. It is expectable that hydroxylated species absorb light more strongly in the longer wavelengths, which may result in rupture of the aromatic ring and in the fast photodegradation of the hydroxyl substituted compounds (Hardy 2002).

To evaluate the role of hydroxyl radical on photodegradation pathway, experiments were performed with a scavenger of ˙OH (D-Mannitol). Comparing the average percentages (n = 3) of photodegradation of BDE-209 in aqueous solution with and without D-Mannitol (one tailed t-test, confidence level of 95 %), for 60 minutes of irradiation, it can be concluded that the BDE-209 photodegradation percentage is higher in the presence of D-Mannitol than in its absence (figure 29).

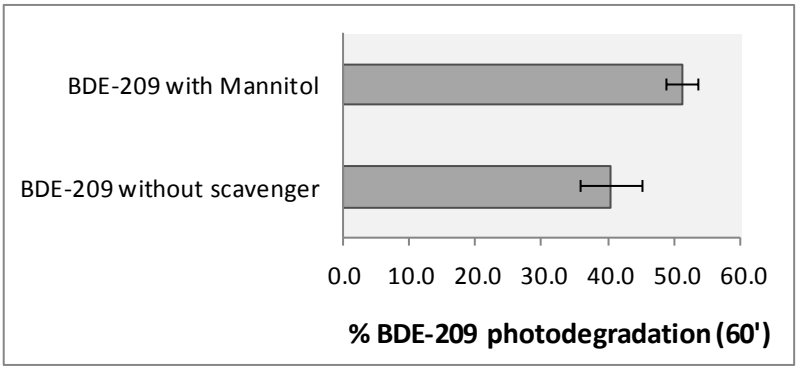


Figure 29 - Influence of D-Mannitol on the percentage of BDE-209 photodegradation after 60' of irradiation.

The presence of the D-mannitol influences not only the photodegradation rate of the BDE-209 but also the formation by-products with chromatographic retention times higher than the retention time of BDE-209 (figure 30). It must be noticed that these experiments were done using DLLME for pre-concentration since it is faster than SPE and it was the same technique used to study the kinetics of degradation in pure water without D-Mannitol. The chromatograms of two solutions with initial identical concentrations of BDE-209, with (chromatogram A) and without (chromatogram B) D-Mannitol, respectively, after 60 minutes of irradiation, are compared in Figure 30. These chromatograms are shown for times higher than 7.5 min, since, as previously referred, this technique is not good to detect the peaks attributed to lower BDEs due to the signals of the blanks in that zone.

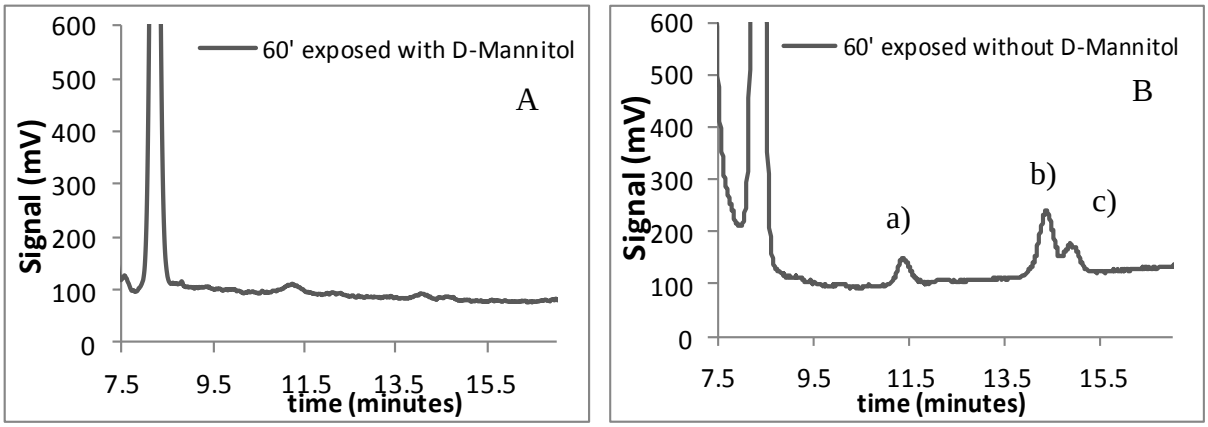


Figure 30 – Chromatograms of the aqueous solutions of BDE-209, after 60 minutes of irradiation, in the presence of D-Mannitol (A) and in the absence of D-Mannitol in aqueous solution. Extraction procedure: DLLME

In figure 30 it is possible to observe that, in the presence of D-Mannitol, the formation of products a), b), c) is inhibited and they are practically absent. Since D-Mannitol is a scavenger for OH radical the first assumption that we could do was that these products were hydroxylated compounds resulting of the reaction with OH radicals. However, taking into account that we are using a reversed phase HPLC column, it was expectable that hydroxylated compounds such as species 4 of figure 28 (more polar than BDE-209) elute before BDE-209. In addition to the routes already mentioned (reaction A and D, figure 28), there are other possible routes which involve reaction between radical species. For instance, Rahman *et al.* (2001) suggest the formation of PBDFs (reaction B, figure 28). The other possibility can involve the formation of dimers (reaction C, figure 28) what would justify some products that elute after BDE-209 (less polar compounds). Based on these considerations, it can be suggested that there are two main routes of by-products formation in the photodegradation of BDE-209. After the formation of species I, the aryl radical (Figure 28), it can give rise to lower BDEs (reaction A) or it can give rise to dimmers or, eventually, PBDFs. In the absence of D-Mannitol the life time of the radical is enough to allow the dimerization to occur since the tendency of water to give hydrogen is quite low. D-Mannitol, as it is an alcohol (figure 31), can act as ethanol, promoting reaction A (Figure 28). It contributes to the increase of the photodegradation rate of the BDE-209 and prevents the formation of products a), b) and c).

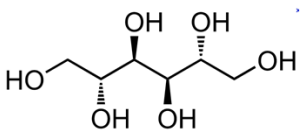


Figure 31 – D-Mannitol

IV. Influence of Humic Sustances on photodegradation

Humic substances are present in great abundance in the environment, namely in the aquatic environment and can affect the photodegradation processes of organic compounds, including pollutants.

Influence of HS on BDE-209 photodegradation in ethanolic solution

The study of HS influence on BDE-209 photodegradative behaviour was made for 5 and 10 minutes of irradiation. The pH and temperature of the solutions were maintained at 4.81 ± 0.70 and 37.2 ± 3.8 º C, respectively.

Figure 32 shows the results of percentage of degradation of BDE-209 in presence of humic acids, fulvic acids and XAD-4 fraction and the means of percentage degradations for each irradiation time were compared by a one way ANOVA. The results shown in tables 9 and 10 allow us to conclude that the BDE-209 photodegradation percentage is significantly decreased in presence of each fraction of HS, for both 5 and 10 minutes of irradiation. This decrease is clearer in the presence of humic acids (HA) and can be due to the higher absorptivity of this fraction of HS, and thus a higher competition for photons in the range of wavelengths where the compound under study absorbs.

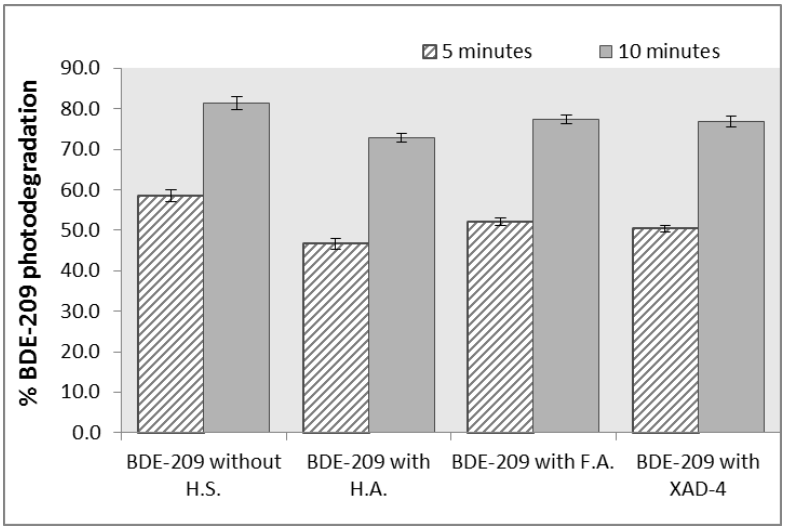


Figure 32 - Influence of HS on percentages of BDE-209 photodegradation in ethanolic solution, after 5 and 10' (n = 3).

Table 9 – ANOVA 1D: photodegradation experiments of BDE-209 with humic substances, at 5'

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	218.60	3	72.86	60.23	7.82E-06	4.07
Within Groups	9.68	8	1.21			
Total	228.28	11				

Table 10 – ANOVA 1D: photodegradation experiments of BDE-209 with humic substances, at 10'

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	108.76	3	36.25	22.62	0.00029	4.07
Within Groups	12.82	8	1.60			
Total	121.57	11				

Figure 33 shows spectra of solutions containing only the different fractions of humics in ethanol, before and after ten minutes of irradiation and one can observe that there are not big differences between before and after the irradiation experiments, which suggests that in this time period the HS do not degrade. This result is according to Farjalla *et al.* (2009) who say that aquatic humic substances present low degradation rates. Note that photodegradation of HS was tested using the same conditions used for BDE-209.

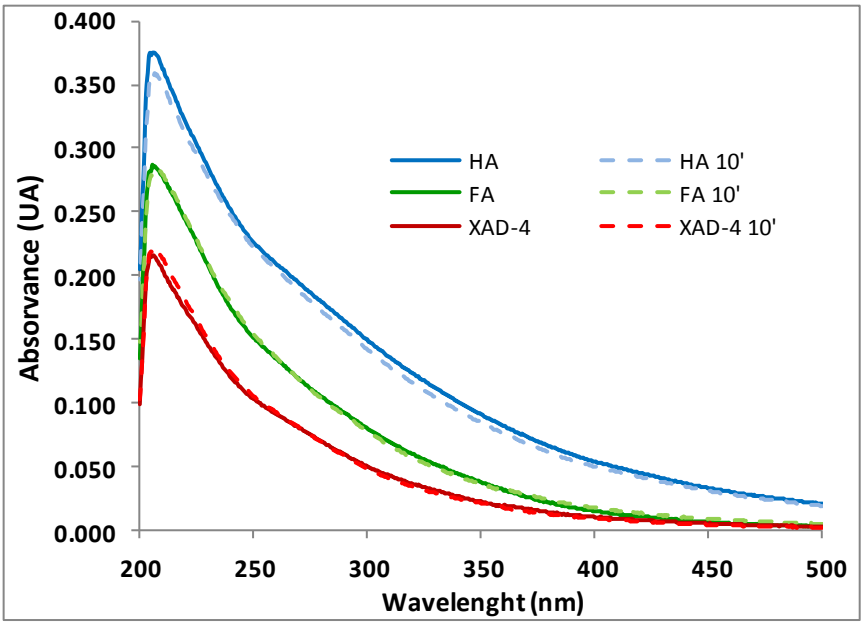


Figure 33 – UV-Vis spectra of HS before (___) and after (_ _ _) 10' of irradiation

As was already referred, humic substances are able to absorb radiation by structures called chromophores (photo-bleaching). Therefore, less radiation is absorbed by molecules of BDE-209 in presence of aquatic HS and consequently, the photodegradation rate is lower.

Direct comparisons of the results obtained to date are not possible because, to our knowledge, there are no studies, particularly with fulvic acids (FA) and XAD-4 fraction. However, Hua *et al.* (2003) irradiated decaBDE adsorbed to HA-coated sand and also found a delay on photodegradation relatively to decaBDE adsorbed to non-coated sand, which is in agreement with our results.

Influence of HS on BDE-209 photodegradation in aqueous solution

The study of the influence of HS on BDE-209 photodegradation in water was tentatively done using DLLME as a pre-concentration procedure. However, after centrifugation, the HS precipitated and it was not possible to remove all organic phase with the micro-syringe (step v. of the DLLME procedure described above, in Materials and Methods).

As an alternative to pre-concentrate BDE-209 in aqueous solutions containing HS, SPE was used. Recovery tests for BDE-209 aqueous solutions without HS, BDE-209 with HA, BDE-209 with FA and BDE-209 with XAD-4 fraction were performed and the results obtained, in percentage, (number of replicates = 3) were 60.94 ± 1.04, 21.60 ± 5.36, 24.10 ± 2.04 and 23.08 ± 6.28, respectively. The recovery percentage of the BDE-209 from aqueous solution without HS is very similar to the value of 57 % obtained by Labadie *et al.* (2010). Looking to recovery percentages of BDE-209 from solutions containing any fraction of HS one can observe that the values of recovery are lower. However, as the degradation percentages are always calculated relatively to the dark controls (light protected solutions inside the solar box together with the irradiated solutions), the recovery will affect the analysis of both protected and exposed solutions in the same way. Thus, we considered that it was possible to compare the photodegradation percentages in the absence and in the presence of HS.

Figure 34 presents the effect of humic substances on BDE-209 photodegradation. The percentage of BDE-209 photodegradation decreased about 30 and 26 % in the presence of HA and FA, respectively. XAD-4 fraction does not significantly influence (t-

test, confidence level of 95 %) on the photodegradation. We already observed the tendency of decrease of photodegradation in experiments in ethanolic solutions; however in aqueous solutions the effect of HS is much higher.

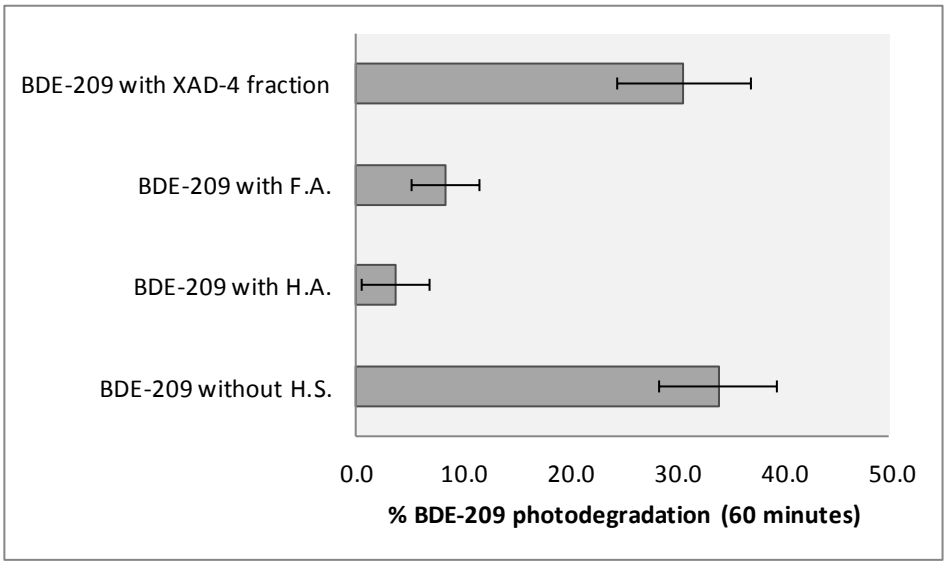


Figure 34 – Influence of HS on percentage of BDE-209 photodegradation, in aqueous solution, after 60 minutes (n = 3) (Leal[b] *et al.* 2012)**.**

The light-shielding effect (Prosen and Zupancic-Kralj 2005), described to explain results in ethanol does not justify the high decrease of photodegradation percentage caused by HA and FA in water at these levels of concentration (8 mg/L). The absorbance obtained for HA, FA and XAD-4 fraction solutions was 0.164, 0.085 and 0.060, respectively (λ = 290 nm, lower limit of the spectral range of irradiation, more close of the maximum of absorbance of BDE-209 – 230 nm) (figure 35).

Other possibility to justify this decrease is the association of BDE-209 to the hydrophobic sites in humic material as has been documented for other organic compounds (Rav-Acha and Rebhun 1992). HA and FA are more hydrophobic than XAD-4 fraction and, thus, the BDE-209 presents a higher tendency to establish a stronger binding to these hydrophobic fractions (HA and FA) than to XAD-4 fraction.

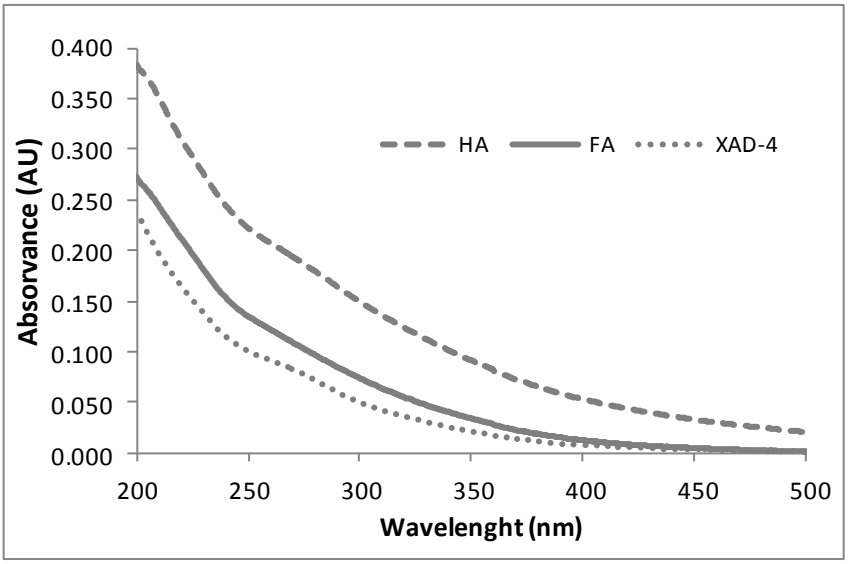


Figure 35 – UV – Vis spectra of HS (8 mg/L, non-irradiated)

The micellar model of humic substances in aqueous solution is also suggested by other authors to justify the delay of photodegradation of other organic contaminants in the presence of humic substances (Wandruszka 1998; Sutton and Sposito 2005; Aleksandrova *et al.* 2011). These authors propose intra or intermolecular organizations to form interior hydrophobic regions separated from aqueous surroundings by hydrophilic exterior. However, at these levels of concentration of HS (8 mg/L), the formation of micellar structures is not expected because the critical micellar concentration (CMC) of the HS is 10 g/L (Conte and Piccolo 2002), but it can cover a range up to much higher values, depending strongly on the type and the nature of HS. Since decaBDE is much more soluble in ethanol than in water, the propensity of BDE-209 for binding to the HS is much lower in ethanol than in water and, thus, the effect of HS in ethanol is probably due only to the light-shielding.

Since in these experiments about the influence of humic substances on photodegradtion were performed using SPE for pre-concentration, we decided to confirm that the results obtained in the absence of HS were consistent with those obtained in the kinetic study performed using DLLME for pre-concentration. Thus, the percentages of photodegradation of BDE-209 after 60 minutes of irradiation determined using DLLME or SPE as pre-concentration procedures were compared and we observed that the mean values (n = 4) were not significantly different (t-test, 95% confidence).

Conclusions

Overview and Future perspectives

Conclusions

Polybrominated flame-retardants are compounds with environmental relevance due to their widespread use, their occurrence in different environmental matrices, their tendency to bioacumulate and their toxic effects. DecaBDE or BDE-209 is one of the most widely used flame retardants and has been recently considered a priority contaminant. Its photodegradation in some organic solvents is documented in the literature and it is known that BDE-209 degrades into less brominated and more toxic compounds. To our knowledge, this is the first study of the kinetics of photodegradation of BDE-209 in aqueous solution (5 μg/L).

To sum up, the present work includes not only a review about several properties of BDE-209 and its environmental relevance but also the results of experimental work obtained. The main results/conclusions are:

- BDE-209 photodegradation in water (0.0101 ± 0.0003 min^{-1}) is 20 times lower than in ethanol (0.208 ± 0.008 min^{-1}).
- In ethanol we detected the formation of several lower brominated compounds.
- In water, contrarily to what is suggested in the literature, we observed that lower brominated compounds can be formed by photodegradation of BDE-209, but four more products were also detected.
- Experiments with aquatic humic substances showed a tendency to the decrease of BDE-209 photodegradation. This decrease is more pronounced in aqueous solution, mainly with the HA and FA fractions. In ethanol, the lag is lower and more evident for the HA fraction.

Other additional conclusions obtained in this work are:

- The LOD achieved with DLLME – HPLC – UV procedure for the analysis of BDE-209 in aqueous solution was 523 ng/L (1 – 5 μg/L).
- DLLME is the better choice for kinetic studies and SPE is more appropriate for the detection of photoproducts.

Overview and Future perspectives

The studies carried out in the present work have allowed achieving new and good results concerning the photodegradation of BDE-209 in water. This study was considered important and simultaneous "practically impossible" by other authors (Erikson *et al*. 2004). Until now, the literature only refers studies of photodegradation in organic solvents or their mixtures with water and speculates about the possible photodegradation routes in water.

Following these good results, an article with the title "BDE-209: Kinetics of photodegradation in water samples" was submitted to the Chemosphere and is under review. DLLME – HPLC – UV procedure was already presented, as an oral communication, at the 7th National Meeting on Chromatography, in Porto. Furthermore, will be presented a poster communication, at the 9th International Symposium on Environmental Geochemistry, to be held in Aveiro between 15 and 21 July.

As proposed at the Seminar of the Chemistry Master Course, the improvement of the analytical method DLLME –HPLC –UV was achieved and its application to the BDE-209 photodegradation in aqueous solution was performed. The study of the interference of humic substances was tested too. In addition, other methodologies as SPE were developed for PBDE pre-concentration and several photodegradation products were detected. Different tests, including tests with a scavenger, allowed understanding a little more about the possible pathways of BDE-209 photodegradation.

In terms of future prospects, the priority is the characterization of the photoproducts of BDE-209 photodegradation by mass spectrometry. Additionally, the prospects may also include recovery tests in river and estuarine waters as well as the continuation of tests of the interference of some components of natural waters on the pre-concentration procedures. The improvement of the method of analysis of BDE-209, including new methodologies of pre-concentration, will be done in order to achieve lower detection limits and to apply it to the analysis of BDE-209 in effluents of some wastewater treatment plants (ETARs) from the region.

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Annex 1

(Brooke *et al.* 2009)

BDE-1	2-Bromodiphenyl ether
BDE-2	3-Bromodiphenyl ether
BDE-3	4-Bromodiphenyl ether
BDE-4	2,2'-Dibromodiphenyl ether
BDE-5	2,3-Dibromodiphenyl ether
BDE-6	2,3'-Dibromodiphenyl ether
BDE-7	2,4-Dibromodiphenyl ether
BDE-8	2,4'-Dibromodiphenyl ether
BDE-9	2,5-Dibromodiphenyl ether
BDE-10	2,6-Dibromodiphenyl ether
BDE-11	3,3'-Dibromodiphenyl ether
BDE-12	3,4-Dibromodiphenyl ether
BDE-13	3,4'-Dibromodiphenyl ether
BDE-14	3,5-Dibromodiphenyl ether
BDE-15	4,4'-Dibromodiphenyl ether
BDE-16	2,2',3-Tribromodiphenyl ether
BDE-17	2,2',4-Tribromodiphenyl ether
BDE-18	2,2',5-Tribromodiphenyl ether
BDE-19	2,2',6-Tribromodiphenyl ether
BDE-20	2,3,3'-Tribromodiphenyl ether
BDE-21	2,3,4-Tribromodiphenyl ether
BDE-22	2,3,4'-Tribromodiphenyl ether
BDE-23	2,3,5-Tribromodiphenyl ether
BDE-24	2,3,6-Tribromodiphenyl ether
BDE-25	2,3',4-Tribromodiphenyl ether
BDE-26	2,3',5-Tribromodiphenyl ether
BDE-27	2,3',6-Tribromodiphenyl ether
BDE-28	2,4,4'-Tribromodiphenyl ether
BDE-29	2,4,5-Tribromodiphenyl ether
BDE-30	2,4,6-Tribromodiphenyl ether
BDE-31	2,4',5-Tribromodiphenyl ether
BDE-32	2,4',6-Tribromodiphenyl ether
BDE-33	2,3',4'-Tribromodiphenyl ether

BDE-34	2,3',5'-Tribromodiphenyl ether
BDE-35	3,3',4-Tribromodiphenyl ether
BDE-36	3,3',5-Tribromodiphenyl ether
BDE-37	3,4,4'-Tribromodiphenyl ether
BDE-38	3,4,5-Tricholodiphenyl ether
BDE-39	3,4',5-Tribromodiphenyl ether
BDE-40	2,2',3,3'-Tetrabromodiphenyl ether
BDE-41	2,2',3,4-Tetrabromodiphenyl ether
BDE-42	2,2',3,4'-Tetrabromodiphenyl ether
BDE-43	2,2',3,5-Tetrabromodiphenyl ether
BDE-44	2,2',3,5'-Tetrabromodiphenyl ether
BDE-45	2,2',3,6-Tetrabromodiphenyl ether
BDE-46	2,2',3,6'-Tetrabromodiphenyl ether
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether
BDE-48	2,2',4,5-Tetrabromodiphenyl ether
BDE-49	2,2',4,5'-Tetrabromodiphenyl ether
BDE-50	2,2',4,6-Tetrabromodiphenyl ether
BDE-51	2,2',4,6'-Tetrabromodiphenyl ether
BDE-52	2,2',5,5'-Tetrabromodiphenyl ether
BDE-53	2,2',5,6'-Tetrabromodiphenyl ether
BDE-54	2,2',6,6'-Tetrabromodiphenyl ether
BDE-55	2,3,3',4-Tetrabromodiphenyl ether
BDE-56	2,3,3',4'-Tetrabromodiphenyl ether
BDE-57	2,3,3',5-Tetrabromodiphenyl ether
BDE-58	2,3,3',5'-Tetrabromodiphenyl ether
BDE-59	2,3,3',6-Tetrabromodiphenyl ether
BDE-60	2,3,4,4'-Tetrabromodiphenyl ether
BDE-61	2,3,4,5-Tetrabromodiphenyl ether
BDE-62	2,3,4,6-Tetrabromodiphenyl ether
BDE-63	2,3,4',5-Tetrabromodiphenyl ether
BDE-64	2,3,4',6-Tetrabromodiphenyl ether
BDE-65	2,3,5,6-Tetrabromodiphenyl ether
BDE-66	2,3',4,4'-Tetrabromodiphenyl ether
BDE-67	2,3',4,5-Tetrabromodiphenyl ether
BDE-68	2,3',4,5'-Tetrabromodiphenyl ether
BDE-69	2,3',4,6-Tetrabromodiphenyl ether
BDE-70	2,3',4',5-Tetrabromodiphenyl ether

BDE-71	2,3',4',6-Tetrabromodiphenyl ether
BDE-72	2,3',5,5'-Tetrabromodiphenyl ether
BDE-73	2,3',5',6-Tetrabromodiphenyl ether
BDE-74	2,4,4',5-Tetrabromodiphenyl ether
BDE-75	2,4,4',6-Tetrabromodiphenyl ether
BDE-76	2,3',4',5'-Tetrabromodiphenyl ether
BDE-77	3,3',4,4'-Tetrabromodiphenyl ether
BDE-78	3,3',4,5-Tetrabromodiphenyl ether
BDE-79	3,3',4,5'-Tetrabromodiphenyl ether
BDE-80	3,3',5,5'-Tetrabromodiphenyl ether
BDE-81	3,4,4',5-Tetrabromodiphenyl ether
BDE-82	2,2',3,3',4-Pentabromodiphenyl ether
BDE-83	2,2',3,3',5-Pentabromodiphenyl ether
BDE-84	2,2',3,3',6-Pentabromodiphenyl ether
BDE-85	2,2',3,4,4'-Pentabromodiphenyl ether
BDE-86	2,2',3,4,5-Pentabromodiphenyl ether
BDE-87	2,2',3,4,5'-Pentabromodiphenyl ether
BDE-88	2,2',3,4,6-Pentabromodiphenyl ether
BDE-89	2,2',3,4,6'-Pentabromodiphenyl ether
BDE-90	2,2',3,4',5-Pentabromodiphenyl ether
BDE-91	2,2',3,4',6-Pentabromodiphenyl ether
BDE-92	2,2',3,5,5'-Pentabromodiphenyl ether
BDE-93	2,2',3,5,6-Pentabromodiphenyl ether
BDE-94	2,2',3,5,6'-Pentabromodiphenyl ether
BDE-95	2,2',3,5',6-Pentabromodiphenyl ether
BDE-96	2,2',3,6,6'-Pentabromodiphenyl ether
BDE-97	2,2',3,4',5'-Pentabromodiphenyl ether
BDE-98	2,2',3,4',6'-Pentabromodiphenyl ether
BDE-99	2,2',4,4',5-Pentabromodiphenyl ether
BDE-100	2,2',4,4',6-Pentabromodiphenyl ether
BDE-101	2,2',4,5,5'-Pentabromodiphenyl ether
BDE-102	2,2',4,5,6'-Pentabromodiphenyl ether
BDE-103	2,2',4,5',6-Pentabromodiphenyl ether
BDE-104	2,2',4,6,6'-Pentabromodiphenyl ether
BDE-105	2,3,3',4,4'-Pentabromodiphenyl ether
BDE-106	2,3,3',4,5-Pentabromodiphenyl ether
BDE-107	2,3,3',4',5-Pentabromodiphenyl ether
BDE-108	2,3,3',4,5'-Pentabromodiphenyl ether
BDE-109	2,3,3',4,6-Pentabromodiphenyl ether

BDE-110	2,3,3',4',6-Pentabromodiphenyl ether
BDE-111	2,3,3',5,5'-Pentabromodiphenyl ether
BDE-112	2,3,3',5,6-Pentabromodiphenyl ether
BDE-113	2,3,3',5',6-Pentabromodiphenyl ether
BDE-114	2,3,4,4',5-Pentabromodiphenyl ether
BDE-115	2,3,4,4',6-Pentabromodiphenyl ether
BDE-116	2,3,4,5,6-Pentabromodiphenyl ether
BDE-117	2,3,4',5,6-Pentabromodiphenyl ether
BDE-118	2,3',4,4',5-Pentabromodiphenyl ether
BDE-119	2,3',4,4',6-Pentabromodiphenyl ether
BDE-120	2,3',4,5,5'-Pentabromodiphenyl ether
BDE-121	2,3',4,5',6-Pentabromodiphenyl ether
BDE-122	2,3,3',4',5'-Pentabromodiphenyl ether
BDE-123	2,3',4,4',5'-Pentabromodiphenyl ether
BDE-124	2,3',4',5,5'-Pentabromodiphenyl ether
BDE-125	2,3',4',5',6-Pentabromodiphenyl ether
BDE-126	3,3',4,4',5-Pentabromodiphenyl ether
BDE-127	3,3',4,5,5'-Pentabromodiphenyl ether
BDE-128	2,2',3,3',4,4'-Hexabromodiphenyl ether
BDE-129	2,2',3,3',4,5-Hexabromodiphenyl ether
BDE-130	2,2',3,3',4,5'-Hexabromodiphenyl ether
BDE-131	2,2',3,3',4,6-Hexabromodiphenyl ether
BDE-132	2,2',3,3',4,6'-Hexabromodiphenyl ether
BDE-133	2,2',3,3',5,5'-Hexabromodiphenyl ether

BDE-134	2,2',3,3',5,6-Hexabromodiphenyl ether
BDE-135	2,2',3,3',5,6'-Hexabromodiphenyl ether
BDE-136	2,2',3,3',6,6'-Hexabromodiphenyl ether
BDE-137	2,2',3,4,4',5-Hexabromodiphenyl ether
BDE-138	2,2',3,4,4',5'-Hexabromodiphenyl ether
BDE-139	2,2',3,4,4',6-Hexabromodiphenyl ether
BDE-140	2,2',3,4,4',6'-Hexabromodiphenyl ether
BDE-141	2,2',3,4,5,5'-Hexabromodiphenyl ether
BDE-142	2,2',3,4,5,6-Hexabromodiphenyl ether
BDE-143	2,2',3,4,5,6'-Hexabromodiphenyl ether
BDE-144	2,2',3,4,5',6-Hexabromodiphenyl ether
BDE-145	2,2',3,4,6,6'-Hexabromodiphenyl ether
BDE-146	2,2',3,4',5,5'-Hexabromodiphenyl ether
BDE-147	2,2',3,4',5,6-Hexabromodiphenyl ether
BDE-148	2,2',3,4',5,6'-Hexabromodiphenyl ether
BDE-149	2,2',3,4',5',6-Hexabromodiphenyl ether
BDE-150	2,2',3,4',6,6'-Hexabromodiphenyl ether
BDE-151	2,2',3,5,5',6-Hexabromodiphenyl ether
BDE-152	2,2',3,5,6,6'-Hexabromodiphenyl ether
BDE-153	2,2',4,4',5,5'-Hexabromodiphenyl ether
BDE-154	2,2',4,4',5,6'-Hexabromodiphenyl ether
BDE-155	2,2',4,4',6,6'-Hexabromodiphenyl ether
BDE-156	2,3,3',4,4',5-Hexabromodiphenyl ether
BDE-157	2,3,3',4,4',5'-Hexabromodiphenyl ether
BDE-158	2,3,3',4,4',6-Hexabromodiphenyl ether
BDE-159	2,3,3',4,5,5'-Hexabromodiphenyl ether
BDE-160	2,3,3',4,5,6-Hexabromodiphenyl ether
BDE-161	2,3,3',4,5',6-Hexabromodiphenyl ether
BDE-162	2,3,3',4',5,5'-Hexabromodiphenyl ether
BDE-163	2,3,3',4',5,6-Hexabromodiphenyl ether
BDE-164	2,3,3',4',5',6-Hexabromodiphenyl ether
BDE-165	2,3,3',5,5',6-Hexabromodiphenyl ether
BDE-166	2,3,4,4',5,6-Hexabromodiphenyl ether
BDE-167	2,3',4,4',5,5'-Hexabromodiphenyl ether
BDE-168	2,3',4,4',5',6-Hexabromodiphenyl ether
BDE-169	3,3',4,4',5,5'-Hexabromodiphenyl ether
BDE-170	2,2',3,3',4,4',5-Heptabromodiphenyl ether
BDE-171	2,2',3,3',4,4',6-Heptabromodiphenyl ether
BDE-172	2,2',3,3',4,5,5'-Heptabromodiphenyl ether

BDE-173	2,2',3,3',4,5,6-Heptabromodiphenyl ether
BDE-174	2,2',3,3',4,5,6'-Heptabromodiphenyl ether
BDE-175	2,2',3,3',4,5',6-Heptabromodiphenyl ether
BDE-176	2,2',3,3',4,6,6'-Heptabromodiphenyl ether
BDE-177	2,2',3,3',4,5',6'-Heptabromodiphenyl ether
BDE-178	2,2',3,3',5,5',6-Heptabromodiphenyl ether
BDE-179	2,2',3,3',5,6,6'-Heptabromodiphenyl ether
BDE-180	2,2',3,4,4',5,5'-Heptabromodiphenyl ether
BDE-181	2,2',3,4,4',5,6-Heptabromodiphenyl ether
BDE-182	2,2',3,4,4',5,6'-Heptabromodiphenyl ether
BDE-183	2,2',3,4,4',5',6-Heptabromodiphenyl ether
BDE-184	2,2',3,4,4',6,6'-Heptabromodiphenyl ether
BDE-185	2,2',3,4,5,5',6-Heptabromodiphenyl ether
BDE-186	2,2',3,4,5,6,6'-Heptabromodiphenyl ether
BDE-187	2,2',3,4',5,5',6-Heptabromodiphenyl ether
BDE-188	2,2',3,4',5,6,6'-Heptabromodiphenyl ether
BDE-189	2,3,3',4,4',5,5'-Heptabromodiphenyl ether
BDE-190	2,3,3',4,4',5,6-Heptabromodiphenyl ether
BDE-191	2,3,3',4,4',5',6-Heptabromodiphenyl ether
BDE-192	2,3,3',4,5,5',6-Heptabromodiphenyl ether
BDE-193	2,3,3',4',5,5',6-Heptabromodiphenyl ether
BDE-194	2,2',3,3',4,4',5,5'-Octabromodiphenyl ether
BDE-195	2,2',3,3',4,4',5,6-Octabromodiphenyl ether
BDE-196	2,2',3,3',4,4',5,6'-Octabromodiphenyl ether
BDE-197	2,2',3,3',4,4',6,6'-Octabromodiphenyl ether
BDE-198	2,2',3,3',4,5,5',6-Octabromodiphenyl ether
BDE-199	2,2',3,3',4,5,5',6'-Octabromodiphenyl ether
BDE-200	2,2',3,3',4,5,6,6'-Octabromodiphenyl ether
BDE-201	2,2',3,3',4,5',6,6'-Octabromodiphenyl ether
BDE-202	2,2',3,3',5,5',6,6'-Octabromodiphenyl ether
BDE-203	2,2',3,4,4',5,5',6-Octabromodiphenyl ether
BDE-204	2,2',3,4,4',5,6,6'-Octabromodiphenyl ether
BDE-205	2,3,3',4,4',5,5',6-Octabromodiphenyl ether
BDE-206	2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether
BDE-207	2,2',3,3',4,4',5,6,6'-Nonabromodiphenyl ether
BDE-208	2,2',3,3',4,5,5',6,6'-Nonabromodiphenyl ether
BDE-209	2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether